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ORIGINAL ARTICLES

STUDIES IN INDIAN PULSES

IX. CONTRIBUTIONS TO THE GENETICS OF *MUNG* (*PHASEOLUS RADIATUS* LINN., SYN. *PH. AUREUS* ROXB.)

The inheritance of colour in the flower, unripe pod and in the seed-coat in *mung* and the linkage relations of these characters to other qualitative and quantitative characters

BY

R. D. BOSE,

Superintendent, Botanical Sub-Station, Pusa

(Received for publication on 29th June 1938)

(With Plate XXVI and one text-figure)

I. Introduction

The mode of inheritance of the colour of the flower and of the seed-coat has been studied by several investigators in a number of species of *Phaseolus*, *viz.*, in the common garden bean (*Ph. vulgaris*), in the Adzuki bean (*Ph. chrysanthos*), and the runner bean (*Ph. multiflorus*). The literature on the genetics of *Phaseolus* has been thoroughly reviewed by Kooiman [1931] and by Matsuura [1929] and hence no attempt has been made in this paper to recapitulate the same. Genetical studies in the green gram or *mung* (*Ph. radiatus* Linn.) do not appear to have been reported up to now.

The purpose of this paper is to present the results of an investigation, conducted in the Botanical Section of the Imperial Agricultural Research Institute, Pusa, on the inheritance of colour in the flower, unripe pod and seed-coat and the relationship of these characters among themselves and with certain quantitative characters, in a cross between a green-seeded and a yellow-seeded type of *mung*. It may be pointed out that the yellow-seeded types of *mung* usually fetch a higher price in the market but are mostly indifferent yielders, whereas the green-seeded varieties are usually very prolific. As a result of the present hybridisation work a number of high-yielding, yellow-seeded hybrids have been secured.

II. Material and method

Green gram or *mung* is one of the most nutritious of pulses, and in common with the other members of the *Leguminosae*, it forms an important crop in the rural economy of India. Forty different types of this crop, comprising four distinct varieties, have been isolated and described by Bose [1932] and a study of the root-system of all these types and of twenty-five types of the allied species, black gram, *Phaseolus mungo*, var. *Roxburghii* has been published by Bose and Joglekar [1933].

A cross was made in 1932 between Pusa Type 18 (dark-green seeded *mung* belonging to the variety *typica*) and Pusa Type 36 (a pale lemon-yellow seeded type falling under the variety *aureus*). The reciprocal cross was also attempted at the same time but no seeds were obtained.

Though hundreds of flowers were crossed, only one fertile pod was obtained. The *mung* flower is very delicate and sheds readily ; this probably is the reason for the poor setting obtained. Of course the hot dry weather prevailing at the time when the crop is in full bloom at Pusa, *viz.*, in March and April, may be another factor which contributes also to the failure in securing good setting.

The pollen is usually shed before the opening of the flowers and for the purposes of hybridisation emasculation should be done as soon as the buds are nearly full grown, but before the standards begin to open. The best rule appears to be to emasculate the buds one or two days before the anthers are mature and then to pollinate the flowers a day or two after this operation. Fertilisation is usually complete in a couple of days, when the growing pod may be seen emerging out of the floral envelope.

For purposes of comparison pure seeds of the parental types were always grown along with the different filial generations. In this experiment only one particular field (Northern Trial Ground No. 11) was used for three consecutive years. No manures were applied to the *mung* itself but this crop invariably followed paddy which had received a dressing of niciphos at the time of its transplantation. *Mung* was sown in or about the second week of February and was usually harvested by the following May or June.

F_1 Generation

Only two F_1 plants were obtained and were grown in the field in 1933. In Table I are presented some of the important characters of the F_1 plants as well as of the two parents.

Table I

Some differentiating characters in Pusa mung *Types 18 and 36 and in the F_1 hybrid plants observed in 1933*

Characters	Type 18	F_1 hybrids	Type 36
1. Habit . .	Semi-erect .	Semi-erect but with a tendency towards erectness.	Semi-erect
2. Maturity as measured by the average number of days taken to flower	Early (74 days) .	Very early (56 days)	Early (65 days)

TABLE I—*contd.*

Characters	Type 18	F_1 hybrids	Type 36
3. Stem colour .	Purplish green	Green with purplish splashes	Green with purplish splashes
4. Leaves . .	Green, medium in size	Green, medium in size	Light green, medium in size
5. Flower colour .	Light yellowish olive	Intermediate light yellowish olive	Olive yellow
6. Pod colour—unripe	Dark green with deep red veins on the sutures.	Green with red veins on the sutures	Green with no red veins on the sutures
7. Pod colour—ripe	Olive grey .	Olive grey	Olive grey
8. Average length of pod in cm.	6·33 ± 0·05	7·05 ± 0·13	7·30 ± 0·05
9. Average diameter of pods in mm.	3·42 ± 0·04	3·61 ± 0·03	3·81 ± 0·03
10. Seed colour and nature of seed-surface	Dark green, dull	Dark green, dull .	Pale lemon-yellow, shining

No clear-cut differences could be seen in the F_2 and F_3 plants in regard to habit, stem-colour and the size of the leaves. The foliage colour also was difficult to distinguish, the development of colour depending to a large extent on the environment and especially on the presence of patches of differential fertility in the field. These characters were not studied, therefore, in any detail. The colour of the ripe pods remained pure for olive grey and showed no segregation. The genic analysis was, therefore, confined to the colour of the flowers, the colour of the unripe pods and that of the seed-coat, and the nature of the seed surface, *i.e.*, whether the seed-coat was shining or dull. Three quantitative characters were studied, namely, maturity (as measured by the number of days taken by the first flower to make its appearance in each plant), the length and the diameter of the pods. The relationship of the colour characters to the quantitative characters just mentioned was also determined. It will be observed from Table I that the colour of the flowers, pods and seeds in Type 36 parent was lighter in tint than that in Type 18 parent.

F_2 AND F_3 GENERATIONS

Selfed seeds of the two F_1 plants were dibbled by hand, under field conditions, in rows one-and-a-half feet apart; the plants were spaced nine inches apart in the row. Thus two F_2 progenies were raised in 1934 from the seeds of each of these F_1 plants and these were denominated for convenience as series A and series B respectively. Single plant progenies of sixty-four selected F_2 plants from series B were grown in the F_3 generation in 1935.

III. Inheritance of qualitative characters

(*1*) *Colour of the flower and of the unripe pod*

The study of the flower colour in the present investigation has been restricted to the colour of the standard. Slight variations were observed also in the colour of the wings as well as in the keel, but as the differences were not always clear-cut, a detailed study of these characters was not attempted.

The parental types of *mung*, *i.e.*, Type 18 and Type 36, have light yellow olive and olive yellow flowers respectively, there being more of olive present in the flowers of Type 18 and more of a yellow tinge in Type 36. For naming colours, Ridgway's " Colour Standards and Colour Nomenclature " was used. The flowers in F_1 plants and plants heterozygous for flower colour in the following generations were characterized by the possession of a lighter tint of olive than that found in Type 18. Considerable care had to be taken to differentiate between the flowers of this intermediate phenotype and those of the Type 18 parent.

The colour of the unripe pods appears to be controlled by the same genes which are responsible for the inheritance of the flower colour and hence the behaviour of these two characters in F_2 and F_3 generations has been considered together. As already mentioned, the unripe pods in Type 18 are dark green in colour and possess deep red veins on both the sutures. In Type 36, on the other hand, the pods are green in colour but have no red veins on the sutures. The F_1 and the heterozygotes possess green pods with faint red veins on the sutures. It must be mentioned, that soil fertility greatly influences the colour of the pods and very often in poor patches of soil the dark green colour in the pods of Type 18 is lost altogether and only a pale yellow colour is developed on them. In such cases it becomes difficult to differentiate between the three phenotypes for pod colour. The presence or absence of the red veins on the sutures is then the only criterion for differentiation and has, therefore, been a more suitable index than the colour itself in recognizing the different phenotypes for pod colour.

The following phenotypes and frequencies were obtained for flower and pod colours :—

Table II

F_2 segregation for the inheritance of flower and unripe pod

Series	No. of plants with flower and pod colours like			Total No. of plants	χ^2	$P <$
	Type 18	Intermediate	Type 36			
A	31	64	25	120	1·133	0·50
B	28	67	36	131	1·097	0·50
Total .	59	131	61	251		

The values of χ^2 and P shown in Table II indicate that the observed and expected frequencies are in harmony and that there is only a single factor difference between the colour of the flowers or of unripe pods.

Representing the flower colour and the unripe pod colour phenotype, resembling Type 18 parent, by the genes **OO** (Light yellowish olive flowers and the dark green pods having deep red veins on the sutures) and the Type 36 phenotype by the allelomorph **oo** (Olive yellow flowers and green pods having no red colour on the sutures), the mode of segregation for these two characters may be shown as—

1 **OO** : 2 **Oo** : 1 **oo**

This hypothesis is fully confirmed by the behaviour of the next generation as is shown below :—

Sixty-four families of this cross were studied in the F_3 generation. Of these twelve families each bred true for the Type 18 and Type 36 phenotypes respectively and forty families segregated for both the characters. Table III shows the frequencies observed in these segregating families.

The fit between observed and expected frequencies is very close indeed. This is confirmed by Fig. 1 which shows graphically the observed and expected χ^2 distributions for the forty segregating F_3 families.

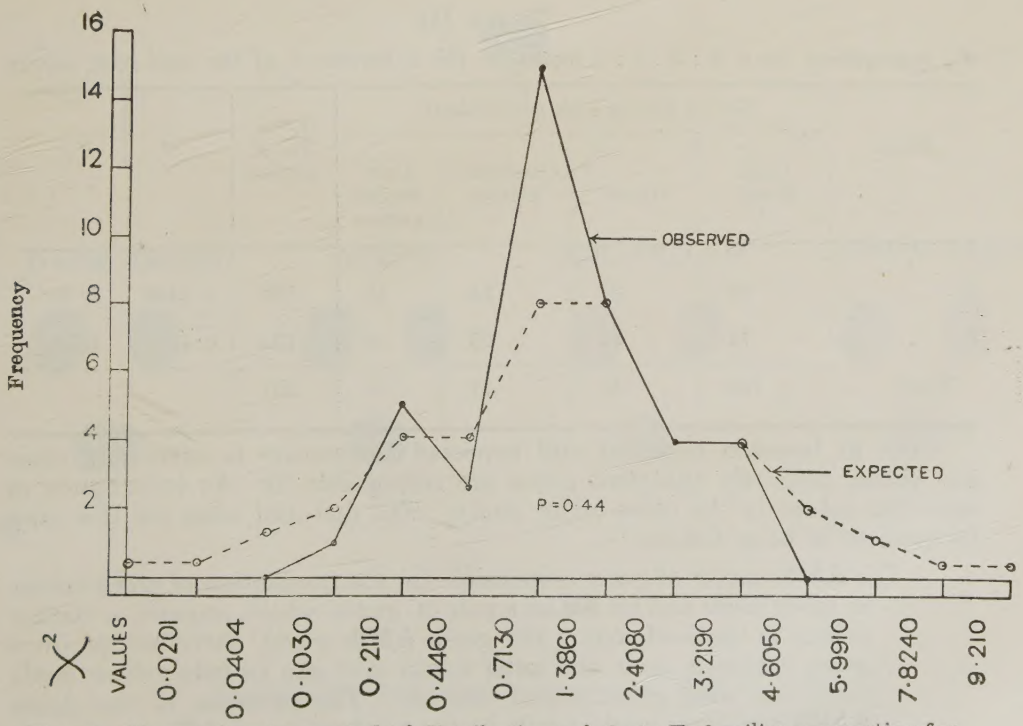

FIG. 1. Observed and expected χ^2 distributions of forty F_3 families segregating for flower and pod colours

It will be observed from Fig. 1 that the deviation between the observed and expected frequencies is insignificant, the value of P being 0·44.

(2) *Inheritance of the seed-coat colour and the nature of the seed surface*

The seed-coat colour in the *mung* cross under investigation may be considered from the economic view-point as the most important character studied and its inheritance has been found to depend on the interaction of two genes as will be described in the following pages. The dull surface of the seed-coat, on the other hand, has been observed to be dominant to shining seed surface, the F_2 showing a clear-cut monogenic type of segregation for the character.

The inheritance of the seed-coat colour and of the seed surface apparently depend on independent genes and no linkage between the two characters has been noted. Similarly there is no linkage between either of these two characters and flower colour.

(*a*) *Seed-coat colour.*—The seeds of Type 18 and those of the F_1 plants were characterised by a dark green colour and a dull surface, while the seeds of the Type 36 parent were of a pale lemon yellow colour and had a shining surface. The F_2 showed the phenotypes and frequencies tabulated below :—

TABLE III

F_2 segregation on a 9 : 3 : 3 : 1 basis for the inheritance of the seed-coat colour

Series	No. of plants with seed colour				Total No. of plants	χ^2	$P <$
	Dark green	Green	Greenish yellow	Pale lemon yellow			
A . .	66	20	24	10	120	1·2440	0·70
B . .	74	25	23	9	131	0·1889	0·95
Total .	140	45	47	19	251		

The fit between observed and expected frequencies is extremely close and shows definitely that two genes are responsible for the inheritance of seed-coat colour in the cross under study. The factorial basis for this may be assumed to be as follows :—

Let **AA** be a pair of genes responsible for the production of green colour in *mung* seeds and let **BB** be a pair of genes which imparts a darker colour to the seed-coat. The genes **AAbb** would, therefore, produce green coloured seeds and **aabb** would give rise to pale yellow seeds (or seeds with green colour absent). The presence of the genes **AABB** together would result in the production of dark green seeds

Inheritance of seed colour in *mung* cross T 18 × T 36

and of the genes **aaBB** would produce greenish yellow seeds. The genic constitution of the Type 18 parent could therefore be represented by **AABB** and that of the Type 36 parent by **aabb**. Four phenotypes were observed in the F_2 in the proportion 9 dark green : 3 green : 3 greenish yellow : 1 pale lemon yellow. This hypothesis was fully substantiated by the results obtained in the F_3 generation.

(*b*) *Nature of the seed surface.*—In respect of the seed surface, the F_2 generation showed the following segregation :—

TABLE IV

F_2 segregation for the nature of the seed surface

Series	Plants with seed surface		Total No. of plants	χ^2	*P*
	Dull	Shining			
A . .	95	25	120	0·5560	0·30
B . .	101	30	131	0·3114	0·50
Total .	196	55	251		

The fit between observed and expected frequencies is close and suggests a single factor difference between dull and shining seeds.

A single factor pair **CC** and **cc** controls the seed surface, and the genic constitution of the parental types in respect of seed colour and seed surface can be represented as follows :—

Type 18 parent . . . **AABBCC**
Type 36 parent . . . **aabbcc**

Considering the seed colour and the seed surface characters together, the segregation in F_2 shows a good fit on the expectation of a 27 : 9 : 9 : 3 : 9 : 3 : 3 : 1 ratio, indicating that the two characters are segregating independently of each other. The actual ratios obtained are presented in Table V given below :—

TABLE V

F_2 segregation on a trihybrid basis for seed-coat colour and the nature of seed surface

Series	F_2 plants with seeds								Total No. of plants	χ^2	*P* <
	Dark green		Green		Greenish-yellow		Pale lemon yellow				
	Dull	Shining	Dull	Shining	Dull	Shining	Dull	Shining			
A .	54	12	14	6	20	4	7	3	120	4·192	0·70
B .	60	14	21	4	15	8	5	4	131	5·853	0·50
Total .	114	26	35	10	35	12	12	7	251		

Even with small F_2 populations of only 120 and 131 plants respectively the fit between observed and expected frequencies on a trihybrid basis is quite close and confirms the fact that there is no linkage between the characters studied. This is further substantiated by the behaviour of the F_3 families.

That the factors for seed-coat colour and flower colour and also for seed surface and flower colour segregate independently of each other is further seen from the segregation shown in Tables VI and VII respectively.

TABLE VI

Relation in F_2 of seed colour to flower colour

Series / Flowers like	No. of plants with seeds												Total No. of plants	χ^2	$P <$
	Dark green			Green			Greenish yellow			Pale lemon yellow					
	T 18	Inter.	T 36	T 18	Inter.	T 36	T 18	Inter.	T 36	T 18	Inter.	T 36			
A	18	34	14	5	12	3	6	12	6	2	6	2	120	2·1	0·99
B	18	39	17	3	13	9	7	9	7	0	6	3	131	7·989	0·70
Total	36	73	31	8	25	12	13	21	13	2	12	5	251		

In spite of the small population clear indications of independent segregation of seed-coat colour on a 9 : 3 : 3 : 1 basis and of flower colour on 1 : 2 : 1 ratio have been obtained.

TABLE VII

Relation in F_2 of the nature of seed surface to flower colour

Series / Flowers like	No. of plants with seed surface						Total No. of plants	χ^2	$P <$
	Dull			Shining					
	T 18	Inter.	T 36	T 18	Inter.	T 36			
A	24	52	19	7	12	6	120	1·498	0·90
B	21	50	30	7	17	6	131	2·213	0·80
Total	45	102	49	14	29	12	251		

Here again it is quite evident that there is no linkage between the seed surface character and flower colour. As the colour of the unripe pod is completely linked with the colour of the flower in this cross, it is obvious that there is no linkage between the colour of the unripe pod and seed colour as well as with seed surface.

This conclusion is supported by the behaviour of the sixty-four families which were studied in detail in the F_3 generation, the full details of which are given in Table VIII.

TABLE VIII

F_3 frequencies for the inheritance of seed colour and the nature of the seed surface as well as of the colour of the flower and of the unripe pods in the same families —mung *Type 18 × Type 36 (1935)*

Family	Seed colour and seed surface												Flower and unripe pod colours					
	F_3 phenotype	F_3 genotype	No. of plants with seed colour and seed surface								Total No. of plants	χ^2	$P <$	No. of plants with flower colour genotype like			Total No. of plants	χ^2	$P <$
			Dark green		Green		Greenish yellow		Pale lemon yellow					Type 18	Intermediate	Type 36			
			Dull	Shining	Dull	Shining	Dull	Shining	Dull	Shining									
B111	Dark green, dull	AABBCC	All	...	...	...	...	...	...	...	All	...	...	All	...	...	All	...	...
B126	„ „ „	„	All	...	...	...	...	...	...	...	All	...	...	...	...	All	All	...	...
B40	„ „ „	„	All	...	...	...	...	...	...	...	All	...	...	19	48	20	87	0·954	0·50
B125	„ „ „	„	All	...	...	...	...	...	...	...	All	...	...	24	53	18	95	2·031	0·30
B115	„ „ „	AABBCc	72	25	...	...	...	...	...	...	97	0·031	0·80	All	...	...	All	...	...
B83	„ „ „	„	70	16	...	...	...	...	...	...	86	1·875	0·10	...	...	All	All	...	...
B38	„ „ „	AABbCC	70	...	24	...	...	...	...	...	94	0·014	0·80	21	54	19	94	2·170	0·30
B51	„ „ „	AaBBCC	79	...	...	...	26	...	...	...	105	0·003	0·96	19	55	28	102	2·216	0·30
B108	„ „ „	„	59	...	...	...	17	...	...	...	76	0·281	0·50	19	33	23	75	1·505	0·30
B76	„ „ „	AABbCc	47	20	17	8	...	...	...	...	92	1·947	0·50	All	...	...	All	...	...
B19	„ „ „	„	61	19	21	11	...	...	...	...	112	2·537	0·30	32	53	25	110	1·036	0·50
B22	„ „ „	„	56	19	14	4	...	...	...	...	93	1·643	0·50	27	46	30	103	1·349	0·50
B29	„ „ „	„	48	20	25	8	...	...	...	...	101	3·819	0·20	33	49	18	100	4·540	0·10
B31	„ „ „	„	39	18	21	6	...	...	...	...	84	3·617	0·30	28	36	20	84	3·238	0·10
B55	„ „ „	„	61	24	24	11	...	...	...	...	120	2·458	0·30	32	55	33	120	0·850	0·50
B49	„ „ „	AaBBCc	36	10	...	...	9	4	...	...	59	1·506	0·50	All	...	...	All	...	...

TABLE VIII—*contd.*

Family	F_2 phenotype	F_3 genotype	Seed colour and seed surface: No. of plants with seed colour and seed surface								Total No. of plants	χ^2	$P <$	Flower and unripe pod colours: No. of plants with flower colour genotype like			Total No. of plants	χ^2	$P <$
			Dark green		Green		greenish yellow		Pale lemon yellow										
			Dull	Shining	Dull	Shining	Dull	Shining	Dull	Shining				Type 18	Intermediate	Type 36			
B114	Dark green, dull	AaBBCc	50	24	...	...	15	6	...	...	95	2·812	0·30	All	...	...	All	...	...
B110	„ „ „	„	40	17	...	...	20	5	...	...	82	2·340	0·50	22	40	20	82	0·146	0·90
B79	„ „ „	AaBbCC	46	...	13	...	17	...	4	...	80	0·752	0·80	25	38	17	80	1·800	0·30
B97	„ „ „	AaBbCc	34	14	13	4	10	3	4	2	84	1·430	0·98	All	...	...	All	...	...
B25	„ „ „	„	38	14	12	5	8	3	4	2	86	2·520	0·90	...	...	All	All	...	...
B2	„ „ „	„	45	15	20	6	18	6	8	3	121	3·140	0·80	33	59	29	121	0·039	0·80
B3	„ „ „	„	39	9	12	6	8	4	2	4	84	3·680	0·80	24	39	21	84	0·643	0·70
B5	„ „ „	„	46	11	20	8	12	5	8	3	113	7·190	0·30	23	57	29	109	0·990	0·50
B8	„ „ „	„	21	6	13	3	12	4	5	1	65	6·610	0·30	20	29	16	65	1·246	0·50
B48	„ „ „	„	31	12	14	7	15	4	6	3	92	6·070	0·50	23	41	25	89	0·640	0·70
B50	„ „ „	„	28	14	15	8	12	4	9	6	96	16·070	0·02	27	48	22	97	0·526	0·70
B75	„ „ „	„	31	15	19	6	11	1	7	3	93	11·450	0·10	20	50	17	87	2·149	0·30
B80	„ „ „	„	31	16	10	5	6	3	5	2	78	6·330	0·50	16	36	26	78	3·025	0·20
B92	„ „ „	„	38	9	14	8	5	4	8	4	90	17·380	0·02	26	41	21	88	0·977	0·50
B32	Dark green, shining	AABBcc	...	All	...	...	...	...	...	...	All	...	...	All	...	...	All	...	...
B34	„ „ „	„	...	All	...	...	...	...	...	...	All	...	...	All	...	...	All	...	...
B91	„ „ „	AABbcc	...	72	...	31	...	...	...	...	103	1·424	0·20	All	...	...	All	...	...
B73	„ „ „	„	...	42	...	20	...	...	...	...	62	1·740	0·10	13	26	21	60	3·199	0·20
B89	„ „ „	„	...	45	...	17	...	...	...	...	62	0·193	0·70	19	28	15	62	1·097	0·50
B99	„ „ „	„	...	51	...	23	...	...	...	...	74	1·456	0·20	23	27	16	66	3·666	0·10

B119	„ „ „	AaBBcc	...	75	...	...	...	26	...	...	101	0·028	0·80	27	51	23	101	0·327	0·80
B63	„ „ „	AaBbcc	...	45	...	14	...	18	...	8	85	2·027	0·70	21	49	15	85	2·735	0·20
B130	Green, dull	AAbbCC	...	...	All	...	...	...	...	...	All	...	...	22	34	16	72	1·222	0·50
B98	„ „	AAbbCc	...	...	75	31	...	...	...	...	106	1·016	0·30	...	...	All	All	...	...
B105	„ „	„	...	...	71	26	...	...	...	...	97	0·168	0·70	...	...	All	All	...	...
B7	„ „	„	...	...	85	25	...	...	...	...	110	0·300	0·50	23	57	29	109	0·900	0·50
B13	„ „	AabbCC	...	...	49	...	...	...	15	...	64	0·080	0·70	All	...	...	All	...	...
B56	„ „	„	...	...	69	...	...	...	18	...	87	0·870	0·30	...	...	All	All	...	...
B16	„ „	AabbCc	...	...	43	19	...	...	14	3	79	3·771	0·20	17	39	23	79	0·924	0·50
...	Green, shining	AAbbcc	(No families realised)																
B45	„ „	Aabbcc	...	...	...	46	...	...	...	22	68	1·960	0·10	...	...	All	All	...	...
B72	„ „	„	...	...	...	74	...	...	...	27	101	0·160	0·50	28	48	25	101	0·428	0·80
B131	„ „	„	...	...	...	69	...	...	...	19	88	0·545	0·30	23	36	14	73	1·120	0·50
B70	Greenish yellow, dull	aaBBCC	...	...	...	...	All	...	...	...	All	...	...	All	...	...	All	...	...
B54	„ „	aaBBCc	...	...	...	...	47	17	...	...	64	0·080	0·70	All	...	...	All	...	...
B71	„ „	„	...	...	...	...	74	19	...	...	93	1·030	0·30	21	52	20	93	1·322	0·50
B122	„ „	aaBbCC	...	...	...	...	55	...	26	...	81	2·176	0·10	...	...	All	All	...	...
B21	„ „	aaBbCc	...	...	...	...	59	17	24	9	109	1·979	0·10	28	56	25	109	0·247	0·80
B30	„ „	„	...	...	...	...	49	14	15	4	82	0·556	0·30	23	35	24	82	1·780	0·30
B121	„ „	„	...	...	...	...	44	17	18	4	83	0·534	0·30	19	46	18	83	1·000	0·50
B128	Greenish yellow, shining	aaBBcc	...	...	...	...	...	All	...	...	All	...	...	...	...	All	All	...	...
B74	„ „ „	aaBbcc	...	...	...	...	...	29	...	8	37	0·220	0·50	13	21	17	41	1·750	0·30
B112	„ „ „	„	...	...	...	...	...	62	...	20	82	0·016	0·90	27	40	14	81	4·185	0·10
...	Pale lemon yellow, dull	aabbCC	(No families realised.)																
B127	„ „ „	aabbCc	...	...	...	...	...	...	66	18	84	0·570	0·30	...	...	All	All	...	...
B116	„ „ „	„	...	...	...	...	...	...	72	17	89	1·640	0·20	26	39	21	86	1·325	0·50
B28	Pale lemon yellow, shining	aabbcc	...	...	...	...	...	...	...	All	All	...	...	...	...	All	All	...	...
B61	„ „ „	„	...	...	...	...	...	...	...	All	All	...	...	...	...	All	All	...	...
B37	„ „ „	„	...	...	...	...	...	...	...	All	All	...	...	22	56	19	97	2·505	0·20
B43	„ „ „	„	...	...	...	...	...	...	...	All	All	...	...	18	30	15	63	0·428	0·80

The behaviour of the sixty-four F_3 families for the qualitative characters studied is clearly brought out in Table VIII. The genotypic results are in harmony with the expectations and prove that the inheritance of the seed-coat colour in this cross is dependent on the interaction of two genes, while that of seed surface and flower colour are monogenic in nature. Each of these characters is also inherited independently.

It is evident from the χ^2 values provided in the table that most of the individual families do not show any significant departure from the expected distribution.

In the segregation for seed colour it is found that the ' dark green ' group is somewhat, though not significantly, deficient in some families. This is possibly due to the fact that the heterozygote is slightly less pigmented than the homozygote and in studying the segregation of factor **B** a few of these have been wrongly included in the recessive class. It may be pointed out that families with the genotypes **AAbbcc** and **aabbCC** were not realised in the F_3, but have since been isolated from the heterozygotes of these groups.

The genetic constitution of the two parental types for the qualitative characters studied may, therefore, be represented as follows :—

Type 18 parent . . . . **AABBCCOO**
Type 36 parent . . . . **aabbccoo**

IV. Inheritance of quantitative characters

The environment usually brings in many complexities in the way of the elucidation of the mode of inheritance of quantitative characters, but given approximately similar sets of soil, cultural and climatic conditions to parental as well as to other filial generations, it is usually possible to obtain reliable indications regarding the inheritance of such characters.

In the studies reported herein the parental types were always grown along with the F_1, F_2 and F_3 generations and the differences in the range of distribution of plants in the parental types for a particular character, in different years, furnished a basis for determining the seasonal effect therein. Thus it will be seen from Table XI that the average diameter of pods in 1933 was definitely lower in both the parents than those observed in the two subsequent years. Hence the diameter of pods observed in the F_1 plants raised in 1933 cannot be compared directly with those observed in the F_2 and F_3 populations grown in 1934 and 1935 respectively. The effect of the season cannot, therefore, be neglected in comparing average values of a particular quantitative character in studies such as these.

(*1*) *Inheritance of maturity as measured by the date of the appearance of the first flower*

Owing to the incidence of desiccating west winds at the time when the *mung* crop comes to maturity, it is not possible to appreciate differences in the maturity of different plants and hence the date of the appearance of the first flower in the plant has been considered to furnish a more reliable index of maturity than the actual date of ripening. The frequency distributions of the parental, F_1, F_2 and F_3 families are presented in Table IX.

TABLE IX

Frequency distribution of the number of days taken to flower in mung

Family	Year	Class centres of No. of days taken to flower												Total No. of plants	Mean
		55	58	61	64	67	70	73	76	79	82	85	88		
Type 18 parent . .	1933	...	...	...	1	3	6	35	16	2	3	...	...	66	73·64
Type 18 parent . .	1934	...	...	...	...	2	6	25	11	2	...	...	...	46	73·31
Type 18 parent . .	1935	...	...	...	...	2	35	50	23	12	1	...	...	123	73·28
Type 36 parent . .	1933	1	3	9	38	17	6	2	...	...	...	...	...	76	64·67
Type 36 parent . .	1934	...	1	15	19	14	1	...	...	...	...	...	...	50	63·94
Type 36 parent . .	1935	1	5	70	28	15	3	...	...	...	...	...	...	122	62·48
Type 18 × Type 36, F_1 .	1933	2	...	...	...	...	...	...	...	...	...	...	...	2	56·00
Type 18 × Type 36, F_2 (Series A).	1934	...	1	4	32	27	29	8	12	6	1	...	...	120	67·67
Type 18 × Type 36, F_2 (Series B).	1934	...	1	6	40	32	28	11	11	2	...	...	...	131	67·83
Type 18 × Type 36, F_3 —															
B 115 . . .	1935	1	30	58	11	1	1	...	...	...	...	...	...	102	60·53
B 116 . . .	1935	...	5	39	44	5	...	...	...	...	...	...	...	93	62·58
B 13 . . .	1935	...	3	32	23	7	...	...	...	...	...	...	...	65	62·57
B 22 . . .	1935	...	...	...	1	20	57	25	4	1	...	...	...	108	70·39
B 29 . . .	1935	...	...	...	1	37	52	7	3	1	...	...	...	101	69·32
B 122 . . .	1935	...	...	...	1	6	30	41	7	4	...	...	...	89	71·99
B 125 . . .	1935	...	...	...	...	1	20	43	20	15	1	...	1	101	74·07
B 126 . . .	1935	...	1	17	39	37	10	5	4	2	1	1	...	117	66·36
B 34 . . .	1935	...	1	3	2	35	42	15	2	1	2	...	...	103	69·36
B 56 . . .	1935	3	8	9	21	45	20	10	6	5	4	1	...	132	67·66
B 63 . . .	1935	...	1	9	17	34	32	15	2	1	3	2	...	116	68·58
B 83 . . .	1935	...	...	1	19	28	30	10	5	3	1	1	...	98	69·05

It is found that the range of distribution in Type 18 extends from sixty four to eighty-two days approximately with a mean of 73·6 days and in Type 36 from fifty-five to seventy-three days with a mean of about 64·6 days The seasonal variation does not appear to influence this character very much. The F_1 plants flowered in fifty-six days showing a definite dominance of earliness. This supports Fisher's theory that a character for which there has been selection for a long time always shows dominance. The range of the F_2 exceeds the combined range of the two parents and definitely suggests transgressive segregation. The distribution of only a dozen homozygous and heterozygous F_3 families has been shown in the table but this character was studied in sixty-four families and definitely indicated that multiple factors were concerned in the inheritance of maturity in the cross under study.

(*2*) *Inheritance of the length of ripe pods*

Although the average length of pods in the two parents differs but slightly and the F_1 plants showed a tendency to have pods of a similar size to those of Type 36, the range of the F_2 progenies showed a clear one-sided transgression towards the smaller pod length. This fact is shown in Table X. The data from the F_3 families also confirmed the conclusion that multiple factors were responsible for the inheritance of this character.

(*3*) *Inheritance of the diameter of pods*

The frequency distributions of the parental and F_2 and F_3 families are presented in Table XI. Multiple factors appear to be responsible for the inheritance of this character also but the transgression is rather one-sided and the frequency of plants with greater diameters than the parental forms is distinctly greater. It is possible that heterosis is responsible for the nature of the transgression shown.

TABLE X

Frequency distributions of the length of ripe pods in mung

Family	Year	Class centres of length of pods in cms.													Total No. of plants	Mean
		F_2 parent	3·0	3·5	4·0	4·5	5·0	5·5	6·0	6·5	7·0	7·5	8·0	8·5	9·0	
Type 18 parent	1933	...	...	...	...	...	...	4	22	23	11	...	1	...	...	60	6·342
Type 18 parent	1934	...	...	...	...	...	4	9	26	28	25	8	...	...	...	100	6·425
Type 18 parent	1935	...	...	...	...	...	...	9	28	31	29	5	...	...	...	102	6·466
Type 36 parent	1933	...	...	...	...	...	...	...	1	3	19	28	9	...	...	60	7·342
Type 36 parent	1934	...	...	...	...	...	...	1	7	18	41	25	3	...	...	95	6·979
Type 36 parent	1935	...	...	...	...	...	...	5	16	19	31	30	8	...	...	109	6·908
Type 18 × Type 36, F_1	1933	...	...	...	...	...	...	...	...	...	2	...	...	...	...	2	7·000
Type 18 × Type 36, F_2 (Series A)	1934	...	...	...	2	3	3	10	21	28	37	14	2	...	...	120	6·496
Type 18 × Type 36, F_2 (Series B)	1934	...	...	1	3	2	8	10	18	41	35	10	3	...	...	131	6·397
Type 18 × Type 36, F_3—																	
B 22	1935	...	...	4	6	4	11	31	37	9	...	...	...	...	...	102	5·510
B 83	1935	...	...	...	...	2	7	18	32	18	1	...	...	...	...	78	5·885
B 115	1935	...	...	...	...	5	11	19	25	33	2	...	...	...	...	95	5·900
B 116	1935	...	...	...	...	6	5	11	24	14	18	1	...	...	...	79	6·089
B 126	1935	...	...	...	...	1	4	7	28	37	18	2	...	...	...	97	6·314
B 125	1935	...	...	...	...	...	...	13	28	29	26	3	...	...	...	99	6·389
B 56	1935	...	...	...	...	...	4	8	11	33	25	1	...	...	...	82	6·427
B 29	1935	...	2	1	2	3	7	23	27	18	12	4	1	...	...	100	5·935
B 13	1935	...	...	...	1	3	6	12	18	12	5	2	1	...	...	60	5·958
B 122	1935	...	...	1	1	1	4	4	12	21	19	12	3	...	...	78	6·532
B 34	1935	...	...	1	3	5	5	13	30	22	14	1	2	...	...	96	6·031
B 63	1935	..	1	1	1	2	4	7	16	17	22	2	1	2	1	77	6·331

Table XI

Frequency distributions of the diameter of ripe pods in mung

Family	Year	Class centres of diameter of pods in cms.										Total No. of plants	Mean
		F_2 parent	0·25	0·28	0·31	0·34	0·37	0·40	0·43	0·46	0·49	0·52	
Type 18 parent . .	1933	...	...	7	28	22	3	...	...	...	...	...	60	0·321
Type 18 parent . .	1934	...	...	...	6	24	60	9	1	...	...	...	100	0·363
Type 18 parent . .	1935	...	...	...	2	36	40	21	3	...	...	...	102	0·366
Type 36 parent . .	1933	...	...	...	12	22	24	2	...	...	...	...	60	0·348
Type 36 parent . .	1934	...	...	...	...	3	13	69	10	...	...	...	95	0·397
Type 36 parent . .	1935	...	...	...	8	10	72	18	1	...	...	...	109	0·368
Type 18 × Type 36—F_1 .	1933	...	...	...	...	...	2	...	...	...	...	...	2	0·370
Type 18 × Type 36—F_2 (Series A)	1934	...	...	1	1	3	12	34	48	20	1	...	120	0·411
Type 18 × Type 36—F_2 (Series B)	1934	...	...	1	2	2	9	23	58	33	2	1	131	0·425
Type 18 × Type 36—F_3—														
B 30	1935	0·37	2	3	15	27	24	2	1	...	...	...	74	0·34
B 130	1935	0·38	...	1	2	5	21	26	18	6	2	2	83	0·40
B 28	1935	0·39	...	2	6	8	30	10	4	...	...	...	60	0·37
B 89	1935	0·40	...	...	...	2	10	22	20	9	2	...	65	0·40
B 5	1935	0·40	...	...	3	20	45	37	5	2	...	...	112	0·38
B 2	1935	0·40	...	...	4	13	35	21	28	2	...	...	103	0·39
B 127	1935	0·40	...	...	2	10	41	24	6	...	...	...	83	0·38
B 116	1935	0·41	...	...	3	5	37	25	7	2	...	...	79	0·38
B 74	1935	0·41	...	...	1	3	19	7	4	...	...	...	34	0·38
B 34 . . .	1935	0·42	...	...	...	3	16	31	35	8	3	...	96	0·41
B 108	1935	0·42	...	...	...	5	10	18	25	16	3	...	77	0·42
B 114	1935	0·42	...	...	...	1	16	39	32	8	1	...	97	0·41

B 119	1935	0·42	...	...	1	11	22	42	18	2	...	...	96	0·39
B 25	1935	0·42	...	1	2	5	13	16	23	14	4	...	78	0·41
B 48	1935	0·42	...	...	...	7	24	25	24	6	2	...	88	0·41
B 49	1935	0·42	...	1	2	5	13	26	4	...	...	...	51	0·38
B 91	1935	0·42	...	...	...	3	14	26	33	21	2	...	99	0·42
B 29	1935	0·43	...	...	6	11	34	29	19	1	...	...	100	0·38
B 56	1935	0·43	...	...	...	3	9	35	31	4	...	...	82	0·41
B 126	1935	0·43	...	1	2	3	14	25	36	14	2	...	97	0·41
B 21	1935	0·43	...	...	9	20	31	34	5	...	...	...	99	0·38
B 54	1935	0·43	...	...	...	5	11	28	15	2	...	...	61	0·40
B 73	1935	0·43	...	...	...	4	4	12	22	15	6	...	63	0·43
B 76	1935	0·43	...	...	...	8	16	26	25	13	1	...	89	0·41
B 31	1935	0·43	...	...	1	7	22	30	22	3	1	1	87	0·40
B 45	1935	0·43	...	1	3	7	16	26	9	3	1	...	66	0·39
B 79	1935	0·43	...	2	5	12	27	16	9	...	...	...	71	0·37
B 80	1935	0·43	1	2	5	6	12	26	18	6	1	...	77	0·39
B 122	1935	0·44	...	...	...	3	14	16	31	12	2	...	78	0·42
B 83	1935	0·44	...	...	1	9	23	36	7	2	...	...	78	0·39
B 32	1935	0·44	1	2	4	10	24	29	17	6	...	...	93	0·39
B 37	1935	0·44	...	2	7	12	34	35	16	1	...	...	107	0·38
B 43	1935	0·44	...	1	5	8	17	24	6	1	...	...	62	0·38
B 3	1935	0·44	...	...	...	4	17	26	33	3	...	...	83	0·41
B 7	1935	0·44	...	...	2	6	41	45	12	...	...	...	106	0·39
B 16	1935	0·44	...	...	2	1	5	12	26	24	3	...	73	0·43
B 40	1935	0·44	...	...	...	6	13	28	29	2	1	...	79	0·40
B 51	1935	0·44	...	...	5	8	27	25	29	7	...	...	101	0·40
B 22	1935	0·45	...	2	3	14	35	38	8	1	...	...	101	0·38
B 125	1935	0·45	...	...	2	5	16	32	42	3	...	...	100	0·40
B 63	1935	0·45	...	...	4	4	17	24	13	13	2	...	77	0·43
B 115	1935	0·45	...	...	...	5	24	34	26	6	...	...	95	0·41

TABLE XI—*contd.*

Family	Year	Class centres of diamater of pods in cms.										Total No. of plants	Mean
		F_2 parent	0·25	0·28	0·31	0·34	0·37	0·40	0·43	0·46	0·49	0·52	
B 131 . . .	1935	0·45	...	...	...	1	12	22	38	6	1	...	80	0·41
B 97	1935	0·45	...	...	2	12	22	21	16	1	...	...	74	0·39
B 72	1935	0·45	...	2	3	4	18	21	34	14	1	...	97	0·40
B 111	1935	0·45	...	...	...	3	6	12	19	12	1	...	53	0·42
B 112	1935	0·45	...	...	1	3	11	18	28	10	1	...	72	0·41
B 8	1935	0·45	...	...	2	2	18	19	19	3	2	...	65	0·40
B 92	1935	0·45	...	...	2	6	16	28	27	6	1	...	86	0·40
B 50	1935	0·46	...	...	...	2	12	26	23	24	1	...	88	0·42
B 70	1935	0·46	...	...	3	10	38	26	22	3	...	...	102	0·39
B 71	1935	0·46	1	2	10	19	21	15	14	2	...	...	84	0·37
B 98	1935	0·46	...	...	...	9	24	22	34	13	...	...	102	0·40
B 121	1935	0·46	1	1	3	16	34	20	7	2	1	...	85	0·38
B 55	1935	0·46	1	3	11	38	38	21	4	2	1	...	119	0·36
B 61	1935	0·46	2	2	8	16	28	35	6	3	2	...	102	0·38
B 99	1935	0·46	...	...	2	6	12	24	18	3	...	...	65	0·39
B 105	1935	0·46	...	1	3	4	18	30	23	14	2	1	96	0·41
B 13	1935	0·47	...	...	...	1	16	17	17	9	...	...	60	0·40
B 38	1935	0·47	1	...	3	6	14	30	28	6	1	...	89	0·40
B 19	1935	0·47	...	1	1	1	7	22	35	34	6	1	108	0·43
B 110	1935	0·48	...	...	1	6	6	22	21	7	3	...	66	0·38
B 75	1935	0·51	...	...	...	3	26	28	20	9	3	...	89	0·40

The frequency distributions of all F_3 families studied for this character have been shown in this table and have been arranged according to the size of the diameter of the pods of the F_2 parents. It will be found that F_2 plants having the same diameter have produced F_3 families which are homozygous as well as heterozygous for the diameter of their pods.

V. Inter-relationship of the characters studied

The colour of the unripe pod has been found to be completely linked with flower colour, and a positive correlation ($r=0\cdot50\pm0\cdot03$) has been found to exist between the length and diameter of ripe pods in this cross, but there is no evidence of any association existing between :—

(1) Maturity and length of pods,
(2) Maturity and diameter of pods,
(3) Maturity and flower colour,
(4) Maturity and seed colour,
(5) Maturity and seed surface,
(6) Length of pod and flower colour,
(7) Length of pod and seed colour,
(8) Length of pod and seed surface,
(9) Diameter of pod and flower colour,
(10) Diameter of pod and seed colour, and
(11) Diameter of pod and seed surface.

An example to show the absence of any relationship between a qualitative and a quantitative character is furnished in Table XII given below :—

Table XII

Relation between maturity and flower colour in F_2

Flower colour like.	No. of days taken by the first flower to appear									Total No. of plants	Mean	S. Dev.
	58	61	64	67	70	73	76	79	82			
Type 18 . .	..	3	15	12	17	3	8	1	..	59	68·53 ±0·58	4·43 ±0·41
Intermediate .	2	6	37	32	26	10	12	5	1	131	68·19 ±0·42	4·81 ±0·30
Type 36 . .	..	1	20	15	14	6	3	2	..	61	68·03 ±0·52	4·10 ±0·37
Total .	2	10	72	59	57	19	23	8	1	251	68·23 ±0·29	4·57 ±0·20

There are no significant differences between the means and standard deviations of the different phenotypes for flower colour, indicating that there is no linkage between the two characters.

VI. Summary and conclusions

Genetical studies in some species of *Phaseolus* such as *Ph. vulgaris*, *Ph. chrysanthos*, *Ph. multiflorus*, etc., have been made by workers abroad but similar studies in *mung* or *Phaseolus radiatus* Linn (syn. *P. aureus* Roxb.) do not appear to have been reported up to now. The mode of inheritance of the colour in the flower, unripe pod, and seed-coat and in the nature of the seed-surface, as well as of quantitative characters such as maturity, length and diameter of pods, and the relation of these characters to each other have been studied at Pusa and described in this paper.

A cross was made between Pusa Type 18 and Type 36 ; the former has dark green, dull seeds, and light yellowish olive flowers while the latter has pale lemon-yellow coloured seeds with a shining surface and olive yellow flowers. Monogenic inheritance has been observed in this cross for flower colour and the nature of the seed surface, olive yellow flowers and shining seed surface (both like those present in Type 36 parent) being recessive. Two genes appear to be responsible for the inheritance of the seed-coat colour.

Transgressive segregation has been noted in respect of the maturity as well as in the length and diameter of the pods. The colour of the unripe pods appears to be controlled by the same genes which are responsible for the inheritance of the flower colour but there is no linkage between seed-coat colour and the nature of the seed surface nor between any of these two characters and flower colour. Neither is there any relationship between any of these qualitative characters with the quantitative characters studied.

A correlation of $r = 0{\cdot}50 \pm 0.03$ has been observed between the length and diameter of pods but no association exists between these two characters and maturity.

The thanks of the author are due to Mr. S. C. Sen (now of the Statistical Section, New Delhi) for making the cross under his direction and to Dr. B. P. Pal, Imperial Economic Botanist, New Delhi, for helpful criticism in the preparation of the manuscript.

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STUDIES ON THE ROOT-ROT DISEASE OF COTTON IN THE PUNJAB

VII. FURTHER INVESTIGATION OF FACTORS INFLUENCING INCIDENCE OF THE DISEASE

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(With two text-figures)

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Introductory

Cotton root-rot is the most destructive disease of this crop in the Punjab ; in the irrigated tracts of the Province it is becoming a limiting factor in cotton growing. The causal organisms *Rhizoctonia bataticola** and *Rhizoctonia solani* attack the underground parts of the plants.

In a previous publication of this series [Vasudeva, 1935], the extent of occurrence, incidence and economic importance of the cotton root-rot disease in the Punjab were described. Since then, further studies of the disease have shown that the activity of the causal fungi is greatly influenced by environmental factors [Vasudeva, 1937]. The work reported in this paper relaten to the investigation of any possible connection of temperature, humidity and physical and chemical composition of the soil with the disease.

**Rhizoctonia bataticola* = C Strains of Haigh = *Macrophomina phaseoli*,*

II. Experimental

The experiments reported in the present paper were conducted in plots which had been under observation for a number of years. The extent of infection of these plots is definitely known, so that the conditions highly suitable for obtaining reliable and comparable data were available.

A. Effect of soil temperature on the incidence of the disease

To determine the effect of soil temperature on the root-rot disease, inoculation experiments were carried out in soil temperature tanks of Wisconsin type [Jones *et al.*, 1926]. The desired temperature was obtained and maintained with reasonable accuracy during the experimental period by regulation of temperature of water in the tanks by means of electric thermostats. Fairly deep metallic containers were used so that the level of the soil may be kept slightly below that of water in the tank. The containers used were cylindrical and of 9½ inches depth so that it was possible to carry cotton plants for a fairly long period. The tanks were run at 25°, 30°, 35° and 40°C. Cotton plants (*Gossypium hirsutum*) of variety 43F, of known age and growing in these containers, were inoculated by root washing method [Vasudeva, 1935] with the causal fungi *Rhizoctonia solani* and *Rhizoctonia bataticola* and placed in the tanks at the temperatures stated above. The controls were similarly treated except that no inoculum was added. The results of three such experiments are given in Table I. The age of the plants in experiments I, II and III was 16, 24 and 30 days, respectively.

Table I

Effect of soil temperature on infection

Temperature (°C.)	Fungus *	Experiment I			Experiment II			Experiment III		
		Total plants	Plants killed	Per cent mortality	Total plants	Plants killed	Per cent mortality	Total plants	Plants killed	Per cent mortality
25	*R. s.*	11	4	36.4	6	2	33.3	7	3	42.9
	R. b.	12	10	83.3	9	8	88.9	6	3	50.0
	Control	12	0	0	10	0	0	6	0	0
30	*R. s.*	11	5	45.5	10	4	40.0	9	8	88.9
	R. b.	8	5	62.5	9	8	88.9	9	6	66.7
	Control	11	0	0	14	0	0	14	0	0
35	*R. s.*	16	14	87.5	8	5	62.5	7	7	100.0
	R. b.	12	10	83.3	13	12	92.3	6	6	100.0
	Control	14	0	0	7	0	0	2	0	0
39	*R. s.*	10	5	50.0	9	2	22.2	10	1	10.0
	R. b.	14	13	92.9	12	12	100.0	5	5	100.0
	Control	12	0	0	9	0	0	7	0	0

* *R. s.* = *Rhizoctonia solani*, *R. b.* = *Rhizoctonia bataticola*.

The results given in Table I show that cotton plants suffer highest mortality from root-rot due to *R. solani* and *R. bataticola* at temperatures of 35° and 39°C. respectively. Although *R. bataticola* is actively parasitic over a range of temperatures varying from 25° to 39°C., there is a fall noticeable in the parasitic activity of *R. solani* above and below 35°C.

In a similar experiment the effect of variation of temperature after inoculation was studied. The inoculated plants were placed in hot water tanks at soil temperatures of 20° and 35°C. The inoculated containers were allowed to stand in these tanks for twenty-four hours after which two pots inoculated with each of the fungi, *i.e., R. solani and R. bataticola*, were transferred from 35° to 20°C. and from 20° to 35°C. An uninoculated control was also transferred simultaneously. Also inoculated and uninoculated plants were kept as checks constantly at 35°C. and 20°C. in order to compare the effect of variation of temperature on infection. The results of one of the typical experiments are summarised in Table II.

TABLE II

Effect of variation of temperature 24 hours after inoculation on the incidence of the disease

Temperature (°C.)		Pot No.		Fungus	Plants inoculated	Total plants killed	Per cent mortality
Before transfer	After transfer	Transferred	Not transferred				
20	20	—	3	*R. bataticola* .	12	9	75·0
20	35	4 & 5	—	*R. bataticola* .	23	19	82·6
20	35	6 & 7	—	*R. solani* . .	25	25	100·0
20	20	—	8	*R. solani* . .	9	1	11·1
20	35	2	—	Control . .	10	0	0 0
20	20	—	1	Control . .	14	0	0·0
35	20	11 & 12	—	*R. bataticola* .	18	15	83·3
35	35	—	13	*R. bataticola* .	10	9	90·0
35	20	14 & 16	—	*R. solani* . .	21	4	19·0
35	35	—	15	*R. solani* . .	13	13	100·0
35	20	10	—	Control . .	13	0	0·0
35	35	—	9	Control . .	13	0	0·0

The results show that in the case of *R. solani* if the temperature is lowered twenty-four hours after inoculation the incidence of the disease is definitely reduced but in the case of *R. bataticola* such a fall in temperature does not materially affect the incidence of the disease.

However when temperature is raised after inoculation from 20° to 35°C. the mortality in the case of both the fungi is almost as high as in controls maintained at 35°C. throughout.

B. Effect of humidity and temperature on the incidence of the disease

A heavily infected plot was selected in the Botanical area and divided into six sub-plots. *Desi* cotton (*Gossypium indicum*) variety Mollisoni 39 was sown in these plots at the optimum time for attack of root-rot. Three plots were covered by means of thatches, and high humidity and low temperature maintained in these plots by addition of water on the thatches at regular intervals throughout the day. The remaining three plots were not covered and served as control. Temperature and humidity records were taken twice a day at 8 A.M. and 5 P.M. both inside the covered and check plots. The mortality counts were taken at weekly intervals. The results of the experiment are given in Table III.

TABLE III

Effect of humidity and temperature on the incidence of root-rot disease

Week ending	Covered plots						Uncovered plots			
	Per cent mortality root-rot	Av. humidity	Av. air temperature (°F.)		Av.* soil temperature (°F.)	Per cent mortality root-rot	Av. humidity	Av. air temperature (°F.)		Av.* soil temperature (°F.)
			8 A.M.	5 P.M.				8 A.M.	5 P.M.	
7th June 1938	0	65·6	88·5	93·9	—	0	54·7	89·8	102·7	93·9
14th June 1938	0	79·5	81·7	88·7	—	0	69·9	84·3	83·3	86·7
21st June 1938	0	76·7	82·7	91·5	—	2·8	71·4	84·3	93·3	88·0
28th June 1938	0	75·2	83·1	92·6	84·8	2·5	67·1	86·6	94·3	92·6
5th July 1938	0	65·1	80·6	97·7	84·4	1·5	46·7	88·4	99·6	94·9
12th July 1938	0	65·2	85·6	98·0	86·2	5·3	52·3	91·0	102·9	96·0
19th July 1938	0	66·7	85·6	95·4	86·0	13·0	55·8	89·1	100·4	99·5
26th July 1938	0	81·5	80·6	88·0	85·9	8·3	75·9	85·7	91·9	96·6
2nd Aug. 1938	0	72·4	95·4	94·6	87·0	3·2	60·9	88·7	99·8	98·8
9th Aug. 1938	0	71·2	83·7	92·8	86·7	1·4	57·6	90·3	99·3	97·4
16th Aug. 1938	0	77·4	84·6	89·4	86·0	0·9	67·5	87·7	94·6	98·8
23rd Aug. 1938	0	77·1	83·4	90·8	85·6	0·9	63·6	86·6	97·1	95·5
30th Aug. 1938	0	87·8	82·8	87·7	84·3	0·0	77·6	84·6	92·1	93·8

* 30 cm. depth.

In the thatched plots no deaths occurred due to root-rot whereas in the uncovered control plots a large number of plants were killed as a result of root-rot attack from the third week of June to the third week of August. High humidity and low temperature in the covered plots appear to be unfavourable for the progress of the disease. It may, however, be mentioned that in the covered plots growth of plants was very poor.

C. Effect of mixed cropping on the incidence of the disease

Effect of mixed cropping was tested in a piece of land heavily infected with the disease. *Desi* cotton variety Mollisoni 39 was sown in rows three feet apart on 20th May, 1938, *i.e.*, the optimum time for the attack of the disease. Sorghum was sown on the same day in between the cotton rows. Root-rot mortality counts along with soil moisture determinations were made at weekly intervals both in the mixed crop and control in which sorghum had not been sown in between the cotton lines. The soil moisture estimations were made in one foot layers upto a depth of three feet. For estimation of moisture individual samples from different parts of the same plot were taken in order to get a fairly respresentative idea of the moisture content of the whole plot. Also air temperature and humidity was noted every day at 8 A.M. and 5 P.M. both inside the mixed crop and cotton.

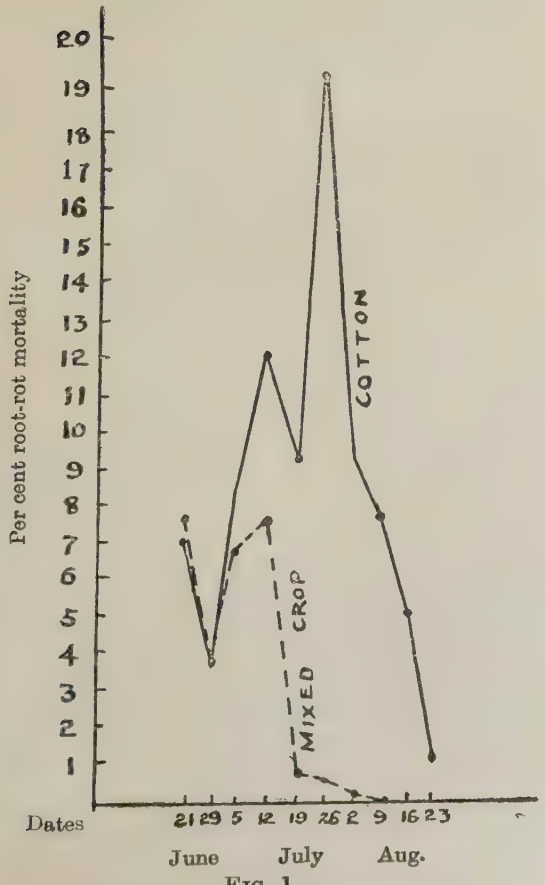

FIG. 1.

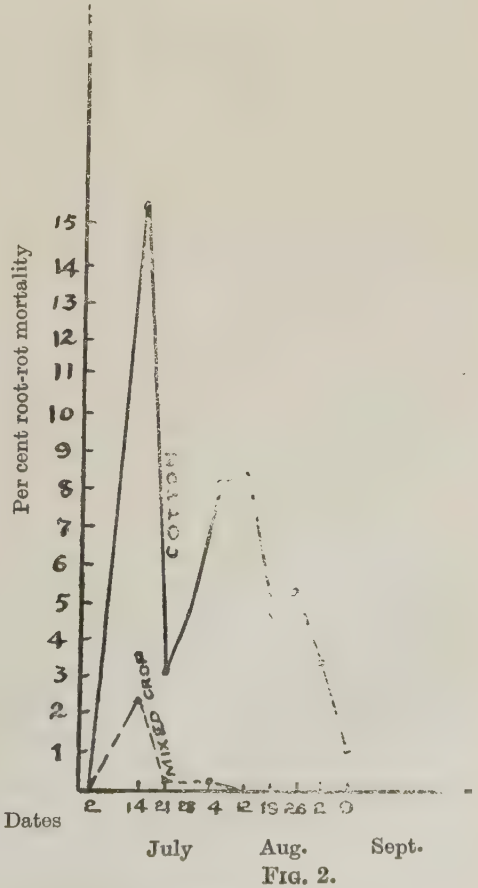

FIG. 2.

Effect of mixed cropping on root-rot mortality

The results of the experiment are set out in Table IV. The soil moisture figures show average percentage of soil moisture in three feet column of soil. Fig. 1 shows the effect of mixed cropping on the incidence of the disease.

The following are the chief points of interest :—

1. Mortality due to root-rot in the mixed crop is significantly lower than in the controls ; t being 2·98 [Fisher, 1925].

2. There is no regular difference in soil moisture content of the inter-cropped and control plots.

3. Humidity is higher in the mixed crop.

4. There is no appreciable difference in air temperature up to 28th June 1938 but during later part of the season air temperature is considerably lower in the mixed crop.

Table IV

Effect of mixed cropping on the incidence of root-rot disease

Week ending	Mixed crop (Cotton + sorghum.)					Cotton (Control)				
	Average per cent mortality root-rot	Average per cent soil moisture	Average per cent humidity	Average Air temperature (°F.)		Average per cent mortality root-rot	Average per cent soil moisture	Average per cent humidity	Average Air temperature (°F)	
				8 A.M.	5 P.M.				8 A.M.	5 P.M.
14th June 1938 .	7·5	—	86·8	85·4	94·1	6·8	—	66·8	85·4	94·1
21st June 1938 .		10·8	81·2	86·0	90·5		10·8	66·8	85·1	96·0
28th June 1938 .	3·4	8·8	67·9	87·4	96·3	3·4	9·7	56·7	87·8	97·8
5th July 1938 .	6·5	13·9	71·3	83·7	93·4	8·2	11·0	47·1	88·8	101·1
12th July 1938 .	7·3	8·4	60·0	89·8	99·7	11·5	10·8	45·2	93·5	105·7
19th July 1938 .	0·6	12·9	67·9	87·8	96·7	8·9	8·8	49·2	90·4	104·7
26th July 1938 .	0·5	10·0	85·2	85·1	90·1	19·1	12·0	76·9	86·4	91·4
2nd August 1938	0·2	8·0	66·7	87·9	98·0	9·0	9·9	53·9	90·4	104·0
9th August 1938 .	0·0	11·9	62·4	87·7	97·1	7·4	12·9	47·1	91·1	105·1
16th August 1938	0·0	8·9	71·1	86·3	92·9	5·0	8·8	65·2	88·0	94·3
23rd August 1938.	0·0	—	76·6	84·3	94·6	1·0	—	60·4	86·9	99·9

Sorghum plants up to 28th June 1938 were very small in size and did not serve as a cover to cotton plants, but as the season advanced there was a marked decline in the number of plants killed due to root-rot in the plots in which cotton was sown in mixture with sorghum. The sorghum plants had then grown tall enough to offer shelter to the cotton plants and were instrumental in lowering air temperature and raising humidity within the crop. It is, however, still a matter for further investigation whether sorghum can also serve as a barrier to the spread of the disease [Jordan *et al.*, 1934].

In another similar experiment sorghum was sown fourteen days before cotton so that sorghum plants could offer protection to cotton plants from the very beginning. Three repeats of both mixed and check plots were kept. Side rows of each plot were left as non-experimental rows. The data of this experiment are given in Table V. Fig. 2 shows the relation of mixed cropping to mortality due to root-rot.

TABLE V

Incidence of the disease in relation to mixed cropping

Week ending	Average per cent root-rot mortality	
	Mixed crop (Cotton+ Sorghum)	Cotton (Check)
2nd July 1938 . . .	0·0	0·3
14th July 1938* . . .	2·3	15·2
21st July 1938 . . .	0·2	3·0
28th July 1938 . . .	0·0	5·0
4th August 1938 . . .	0·1	8·2
12th August 1938 . . .	0·0	8·3
19th August 1938 . . .	0·0	4·8
26th August 1938 . . .	0·0	5·2
2nd September 1938 . .	0.0	3·2
9th September 1938 . .	0·0	0·9
	t = 3·31	

*12 days mortality

The results show that root-rot mortality in the mixed crop is lower from the very beginning of the season and that the results are statistically significant.

D. Effect of soil composition

Root-rot disease of cotton invariably appears in patches. A few green healthy looking plants may be seen scattered all over the infected area, although they are usually plants which have been partially attacked by the disease, but have escaped death by putting out lateral roots in the uppermost layers of soil during wet weather or irrigation.

As healthy and diseased patches appear close to each other, examination of the soil was undertaken. It was considered possible that the information secured might help in finding why the disease causes great damage in one part of the field and does not do so in the adjoining portion.

Soil samples from healthy and disease bearing portions lying side by side in a plot were taken in one foot layers up to a depth of five feet. Samples were taken at Lyallpur, Khanewal and Sargodha. From each of the plots three individual samples were taken up to a depth of five feet and analysed separately, except in the case of Sargodha samples, where representative samples were obtained by thoroughly mixing up the corresponding samples from three different bores made in each healthy and diseased part. The analyses were carried out by the International method [1936].

(*i*) *Mechanical analysis*—Altogether one hundred and thirty samples from Sargodha, Lyallpur and Khanewal were analysed. The results of this analysis in Table VI do not show any regular differences in the texture of healthy and diseased soils.

(*ii*) *Estimation of nitrogen and organic matter.*—Nitrogen was estimated by the A. O. A. C. method and organic matter by Robinson and Mclean's oxidation method [1929]. C : N ratios were calculated from these. Results of these estimations which are given in Table VII do not show regular differences in the nitrogen and organic matter content and C : N ratio of healthy and diseased soil samples.

(*iii*) *Analysis of water extract.*—In this connection forty-eight samples were analysed and estimations were made for the total solids, carbonates, bicarbonates, sulphates, chlorides and calcium. No appreciable differences in any of the constituents estimated were however observed in healthy and diseased soil samples. The results of typical analyses are set out in Table VIII.

TABLE VI

Mechanical analysis of healthy and diseased soil samples

Depth	Sample*	Sargodha. Per cent						Lyallpur† Per cent						Khanewal† Per cent					
		Carbonates	Gravel	Sand	Fine sand	Silt	Clay	Carbonates	Gravel	Sand	Fine sand	Silt	Clay	Carbonates	Gravel	Sand	Fine sand	Silt	Clay
1st foot	H	1·85	0·14	7·70	62·89	16·70	12·68	0·76	—	3·99	69·66	16·86	9·58	5·36	—	2·92	68·30	18·50	10·26
	D	2·70	0·14	5·41	64·13	17·56	12·90	1·04	—	3·65	69·20	12·54	10·15	5·13	—	2·78	70·10	19·73	7·33
2nd foot	H	4·05	0·28	7·68	65·00	14·82	12·50	1·43	—	4·00	73·69	13·03	9·92	4·66	—	2·85	70·06	17·30	10·13
	D	6·15	—	5·61	68·69	14·00	11·60	1·95	—	3·35	72·09	13·71	11·93	4·53	0·12	2·59	73·20	19·10	8·53
3rd foot	H	5·47	0·28	7·78	66·62	14·00	11·60	2·40	—	2·43	72·96	15·02	9·57	4·66	—	3·86	70·50	15·66	9·66
	D	7·87	0·14	4·46	72·58	11·54	10·40	2·83	0·01	3·15	71·60	14·68	10·50	5·26	—	3·56	78·20	9·83	8·33
4th foot	H	6·86	1·68	6·37	68·15	14·09	11·40	2·61	—	2·30	70·57	16·54	10·20	3·86	—	4·72	73·10	13·55	8·65
	D	10·01	0·70	3·50	76·15	12·05	8·30	2·88	—	2·46	71·67	16·19	10·04	6·16	—	4·14	76·60	12·60	6·60
5th foot	H	9·77	14·70	6·50	67·25	15·45	10·80	2·95	0·38	2·37	67·70	20·42	9·46	4·96	2·40	3·47	67·90	19·63	8·93
	D	10·90	4·90	3·99	74·31	12·70	9·00	4·32	3·09	2·22	71·55	16·56	9·37	6·26	—	3·21	78·43	12·20	6·23

*H = Healthy, D = Diseased

†Mean of three samples

Table VII

Distribution of carbon and nitrogen in healthy and diseased soils

Depth	Sample*	Sargodha			Lyallpur†			Khanewal†		
		Per cent nitrogen	Per cent organic matter	C/N	Per cent itrogen	Per cent organic matter	C/N	Per cent nitrogen	Per cent organic matter	C/N
1st foot . . .	H	0·024	0·31	7·50	0·038	0·36	5·48	0·040	0·24	3·44
	D	0·028	0·37	7·80	0·036	0·33	5·50	0·031	0·23	4·47
2nd foot . . .	H	0·035	0·24	3·90	0·010	0·23	13·60	0·024	0·19	6·16
	D	0·024	0·21	5·00	0·019	0·25	11·30	0·028	0·19	3·89
3rd foot . . .	H	0·035	0·25	4·11	0·032	0·20	4·79	0·027	0·26	5·62
	D	0·024	0·23	5·54	0·013	0·24	15·10	0·031	0·16	3·07
4th foot . . .	H	0·028	0·23	4·86	0·016	0·21	7·70	0·027	—	—
	D	0·017	0·18	6·35	0·016	0·20	7·60	0·026	—	—
5th foot . . .	H	0·025	0·22	5·08	0·013	0·23	11·06	0·024	—	—
	D	0·024	0·20	4·79	0·021	0·21	8·60	0·023	—	—

*H = Healthy, D = Diseased

†Mean of three samples

TABLE VIII

Analysis of water extract of healthy and diseased soil samples

Sample		Total solids		Carbonates		Bicarbonate		Sulphates		Chlorides		Calcium	
Depth	No.	H	D	H	D	H	D	H	D	H	D	H	D
1st foot	1	0·056	0·096	*Nil*	*Nil*	0·084	0·100	Traces	*Nil*	0·049	0·084	—	—
	2	0·060	0·079	,,	,,	0·084	0·088	*Nil*	,,	0·052	0·049	—	—
	3	0·048	0·024	,,	,,	0·082	0·092	,,	,,	0·052	0·043	—	—
	4	0·070	0·048	,,	,,	0·092	0·092	,,	,,	0·055	0·032	—	—
2nd foot	1	0·080	0·080	,,	,,	0·065	0·061	—	—	0·095	0·031	0·019	0·022
	2	0·060	0·050	,,	,,	0·068	0·068	—	—	0·038	0·028	0·020	0·019
	3	0·158	0·010	,,	,,	0·076	0·068	—	—	0·039	0·032	0·019	0·017
	4	0·096	0·117	,,	,,	0·070	0·072	—	—	0·028	0·034	0·015	0·018
3rd foot	1	—	0·047	,,	,,	0·070	0·056	0·172	0·020	0·171	0·031	0·036	0·030
	2	0·044	0·054	,,	,,	0·070	0·073	0·012	0·012	0·047	0·034	0·019	0·017
	3	0·048	0·046	,,	,,	0·062	0·063	0·020	0·011	0·025	0·031	0·016	0·017
	4	0·048	0·210	,,	,,	0·079	0·063	0·020	—	0·031	0·041	0·022	0·084
4th foot	1	—	—	,,	,,	0·070	0·055	0·018	0·052	0·060	0·054	0·015	0·018
	2	—	—	,,	,,	0·060	0·068	0·010	0·038	0·050	0·043	0·014	0·015
	3	—	—	,,	,,	0·063	0·063	0·035	0·018	0·033	0·038	0·014	0·014
	4	—	—	,,	,,	0·061	0·045	0·024	—	0·035	0·032	0·014	0·062

(*iv*) *Chemical analysis.*—This included the determination of iron, aluminium, phosphorus, sodium, potassium, calcium and magnesium. Altogether twenty-four samples of diseased and healthy soil were analysed. The analysis did not show any appreciable difference in iron, aluminium, phosphorus, sodium and potassium contents of the healthy and diseased soil samples. Diseased soils, however, contain a higher amount of acid soluble calcium (t=3 and 5) and have a higher Ca : Mg ratio (t=3·5 and 3·2) than the healthy soils. The results of Ca : Mg estimations are given in Table IX.

TABLE IX

Calcium and magnesium contents of healthy and diseased soils (mg. per 100 grms.

	Sample	Healthy			Diseased		
		Ca	Mg	Ca/Mg	Ca	Mg	Ca/Mg
I	1st foot	1050	579	1·81	1322	579	2·28
	2nd foot	1287	1001	1·28	1858	1098	1·69
	3rd foot	1651	1647	1·0	3502	1357	2·58
	4th foot	1851	1454	1·27	4703	1387	3·39
	5th foot	1958	1514	1·29	4253	1261	3·37
II	1st foot	929	645	1·44	1858	585	3·17
	2nd foot	1029	989	1·04	2213	1158	1·91
	3rd foot	1701	1007	1·69	3102	1339	2·32
	4th foot	2251	1128	1·99	5003	1447	3·46
	5th foot	2251	1128	1·99	3902	1062	3·67

Although the roots of diseased plants contain comparatively higher amount of calcium, the percentage of calcium in diseased plants as a whole is lower than in the healthy plants [Vasudeva *et al.*, 1939]. The diseased plant soils, however, contain higher amount of calcium showing thereby that in the diseased plants calcium is not being transported to the upper parts. The picture would have been more complete had it been possible to work out a balance of calcium in the plant, the roots and the soil on which the plant has grown, but this is not possible with plants growing in the field since the soil concerned with the development of any individual plant cannot be considered as an isolated block. Further work in this direction is, however, necessary.

E. Effect of soil reaction

*p*H values of soil samples obtained both from healthy and affected fields were determined electrometrically and were found to vary from 5·4 to 8·6, the range over which the causal fungi, *R. solani* and *R. bataticola*, show good growth. No appreciable differences in *p*H values of healthy and diseased soil samples have however been observed. All the same an attempt was made to test the effect of changing soil reaction in an infected plot on the incidence of the disease. The reaction was changed by the addition of sulphur and lime to the soil and the different treatments were randomised. Six replications of each treatment were made. Lime and sulphur were first applied in 1936 but an additional dose had to be given in 1937 to set up a range of soil reactions varying from *p*H 5 to 8·5.

The data of this experiment showing the variations in soil reaction as a result of the addition of lime and sulphur and the average per cent mortality of cotton plants due to root-rot for three successive years are recorded in Table X. During 1937 germination of cotton seed was very poor in plots treated with sulphur which probably exerted a toxic effect on the seed.

TABLE X

Incidence of the disease in relation to soil reaction

No.	Treatment	1936			1937			1938		
		Dose per acre (lbs.)	*p*H	Average per cent root-rot mortality	Additional dose (lbs.)	*p*H	Average per cent root-rot mortality	Additional dose (lbs.)	*p*H	Average per cent root-rot mortality
1	Sulphur . .	1000	8·3	47·7	3000	7·4	31·4	—	—	56·7
2	Sulphur . .	3000	8·0	40·5	3000	5·9	24·9	—	—	55·7
3	Sulphur . .	4000	7·6	52·6	4000	5·1	30·5	—	—	47·0
4	Lime . . .	1000	8·7	43·4	3000	8·3	35·0	—	—	54·9
5	Lime . . .	2000	8·8	24·3	4000	8·2	31·2	—	—	51·4
6	Untreated Control .	—	8·6	38·1	—	8·2	30·0	—	—	59·1

The results given above indicate that the hydrogen ion concentration of the soil has no effect on the incidence of the disease.

III. ACKNOWLEDGMENTS

Thanks are due to Professor Rai Sahib J. C. Luthra, I. A. S., for valuable criticism and interest throughout the course of this investigation and also to Dr. Ramji Narain for helpful suggestions. Thanks are also due to the Indian Central Cotton Committee for financing the research scheme.

The junior author made some of the field observations under the guidance of the senior author.

IV. Summary

1. The bearing of temperature and humidity on the incidence of root-rot disease of cotton has been shown.

2. Preliminary observations indicate that virulent attack of the disease may be evaded by sowing cotton in mixture with sorghum.

3. There is no difference in the texture and chemical composition of healthy and diseased soils except that the diseased soils contain a higher amount of acid soluble calcium and have a higher Ca : Mg ratio.

4. There is no correlation between the hydrogen ion concentration of the soil and the incidence of the disease.

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THE AMARANTUS BORER, *LIXUS TRUNCATULUS* (F.) AND ITS PARASITES

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(With Plates XXVII and XXVIII)

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I. Introduction

The amarantus borer, as its name implies, bores into stems and branches of various species of cultivated and wild amarantus. Lefroy [1909] recognized, about thirty years ago, that *Lixus truncatulus* breeds freely in plants of amarantus, and Fletcher* [1914] considered it as a minor pest capable of doing considerable damage to cultivated amarantus ; yet very little was known of its life-history and control, while the method of oviposition and the nature and form of egg were altogether unknown.

Plants of the natural order Amarantaceae do not constitute staple crops but several species are cultivated as vegetable, ornamental or medicinal plants in many parts of India. Chief of these are :—

1. *Amarantus gangeticus* Linn.—Cultivated throughout India under the name of *lal sag*. Several varieties form an important vegetable of the country.

2. *Amarantus caudatus* Linn.—Cultivated in the south-western parts of India, also at Pusa. Leaves used as vegetable and the seed, called *ram dana*, employed for medicinal purposes.

Besides these useful species, there are several others such as *Amarantus viridis* Linn. and *Amarantus spinosus* Linn. which grow wild throughout India.

In October 1935, *Amarantus caudatus* growing in the kitchen garden at the Imperial Agricultural Research Institute, Pusa, was found severely attacked by the borer. This gave the writer an opportunity of studying the bionomics and potentialities of this pest. A number of parasites not so far known from this host have been bred from the immature stages of the borer. Some of these seem to be of considerable importance. The habits, status, life-history and morphology of the important ones have been worked out. A preliminary account of this work was communicated to the 24th Session of the Indian Science Congress and a brief account also appeared in the Annual Report of the Imperial Entomologist [Pruthi, 1936].

II. Biology and morphology of *Lixus truncatulus*

Distribution

The borer is widely distributed, occurring almost throughout the plains of India, from the Punjab in the west to Burma in the east and Bihar in the north to Madras in the south. Details of its distribution and food plants of the specimens contained in the Pusa, Coimbatore, Dehra Dun and Calcutta collections are given in Table I. It is clear that the insect occurs all the year round and is more abundant in South India than elsewhere.

*Fletcher records this species under the name *L. brachyrhinus* Bohem., which is now considered to be a synonym of *L. truncatulus* (Fabr.)

TABLE I

Distribution and food plants of Lixus truncatulus

Month	Locality and province	No. of individuals	Food plants and remarks
January	Dehra Dun (U. P.)	Several	*Amarantus spinosus**
	Nagpur (C. P.)	1	....
	Saidapet (Madras)	Several	*Amarantus* sp.*
February	Bombay	2	Wild grass
	Pusa (Bihar)	1	Oat leaf
	Coimbatore (Madras)	..	....
March	Dehra Dun (U. P.)	Several	*Amarantus spinosus**
	Bombay	1	....
	Daltonganj (Bengal)	1	*Zizyphus jujuba*
	Coimbatore (Madras)	..	....
April	Dehra Dun (U. P.)	Several	*Amarantus spinosus**
	Surat (Bombay)	1	....
	Lahore (Punjab)	1	*Amarantus* sp.
	Pusa (Bihar)	5	*Amarantus spinosus**
	Ganjam (S. India)	Several	At light.
	South Arcot (S. India)	..	*Amarantus* sp.
May	Purneah (Bengal)	..	....
	Pusa (Bihar)	1	Indigo
	Pusa (Bihar)	1	*Amarantus* sp.
	Lahore (Punjab)	2	*Amarantus* sp.
	Coimbatore (Madras)	..	....
June	Pusa (Bihar)	1	....
	Coimbatore (Madras)	1	*Babul*
	Madras	Several	*Amarantus gangeticus*
July	Siliguri (Bengal)	..	....
	Calcutta (Bengal)	..	....
	Darjeeling (Bengal)	..	....
	Ballygunj (Bengal)	..	At light
	Mandalay (Burma)	2	*Amarantus* sp.
	Kalka (Punjab)	1	....
	Pusa (Bihar)	3	....
	Coimbatore (Madras)	..	*Babul*
August	Purneah (Bengal)	..	....
	Pusa (Bihar)	2	*Amarantus gangeticus**
	Upper Burma	3	....
	Trichinopoly (Madras)	1	*Amarantus* sp.

* In these cases the immature stages were found actually in the food plants mentioned.

TABLE I—*concld.*

Month	Locality and province	No. of individuals	Food plants and remarks
September .	Dehra Dun (U. P.) . .	Several	*Amatantus gangeticus**
	Monghyr (Bihar) . .	..	....
	Pusa (Bihar) . . .	2	*Amarantus gangeticus* *
	Saidapet and Vizag. (Madras).	7	*Amarantus* sp.
October . .	Dehra Dun (U. P.) . .	..	*Amarantus gangeticus**
	Chingliput (Bombay) .	1	*Amarantus* sp.
	Cawnpore (U. P.) . .	2	Niger
	Surat (Bombay) . .	1	....
	Nagpur (C. P.) . .	1	Cotton
	Bilaspur „ . . .	1	....
	Pusa (Bihar) . . .	4	*Amarantus* sp.*
	Madhopur (Bengal) . .	..	....
	Attur, Annampali, Godavary (S. India).	..	*Amarantus* sp.*
November .	Dehra Dun (U. P.) . .	Several	*Amarantus spinosus**
	Pusa (Bihar) . . .	2	*Cucurbitaceae*
		3	*Amarantus* sp.
	Gurdaspur (Punjab) . .	1	....
	Cuttack (Bengal) . .	1	....
December .	Gorakhpur (U. P.) . .	1	....
	Trichinopoly (Madras) .	1	....
	Godavary (Madras) . .	..	....

Nature of damage and food plants

After hatching under the epidermis of a twig, the young grub bores towards its pith. On reaching the pithy portion in the centre it bores along the length of the branch, the passage of least resistance. It is, however, interesting to find that the grub almost invariably bores towards the proximal end which is the thicker end of the branch. Arriving at the junction it burrows into the next thicker branch; in this way increase in the size of the grub automatically synchronises with increase in the thickness of the branch. When full grown, the grub prepares a wide chamber for pupation. This is marked by a characteristic swelling of the stem externally. In severe cases of attack one may find a large number of such swellings marking the position of full-grown grubs and pupae. From a single large plant of *Amarantus caudatus* 155 individuals of this species in different stages of development were collected. Fletcher recorded only ten to twelve grubs infesting a single host plant. As a result of such a heavy infestation the plant growth becomes

* In these cases the immature stages were found actually in the food plants mentioned.

stunted, and there are but few tender shoots and new leaves left on the plant which are the chief portion used as vegetable. All the old branches and main stem present a twisted and swollen appearance and finally there is very limited flowering and setting of seed.

Though a pest of amarantus, the weevil has also been reported on a variety of other plants, *e.g.*, niger, indigo *Zizyphus jujuba*, *Lagenaria vulgaris*, *Solanum melongena*, etc. It is interesting to note that with the exception of amarantus, the larval stages are not known from any other host plant.

In order to determine the actual food plants of this weevil, fivepairs of adults, emerged between 6th and 9th December, 1935, were caged on different species of *Amarantus* and other allied plants. Before introducing the weevils, the parts of the plant enclosed were examined carefully for eggs and grubs. Twigs showing oviposition or damage, such as nibbled leaves, etc., were rejected. After every twenty-four hours the weevils were transferred to a fresh twig, the previous one being examined for eggs as well as damage to leaves. The experiments repeated six to eleven times with each food plant show that all the four species of *Amarantus*, viz., *gangeticus*, *caudatus*, *viridis* and *spinosus*, are attacked by the weevil, their leaves and tender branches are eaten by the adults, eggs are laid on them and the grubs feed inside their stem. *Amarantus spinosus* is the least susceptible; eggs were laid only in three cases out of eight and the leaves were only slightly nibbled. This is supported by field observations, which show that when other species of *Amarantus* are available, plants of *Amarantus spinosus* in the same locality are quite healthy and free from attack. They are only attacked in case the other varieties are absent or scarce. In the case of *Chenopodium album* the leaves were reluctantly nibbled after some starvation, and eggs were laid in the stem after boring several empty holes. The young grubs in this case were found to develop inside the stem to some extent but could not be reared to the adult stage. It seems that the weevil infests *Chenopodium album* and *Amarantus spinosus* under conditions of scarcity of suitable species of *Amarantus*. The leaves of *Zizyphus jujuba* were slightly nibbled by the adult weevils but neither eggs were laid on it nor were the grubs found inside the stem. On *Lagenari vulgaris* and *Solanum melongena* the weevils could neither feed nor oviposit.

Emergence, copulation and pre-oviposition period

This weevil is a rather slow-developing insect with overlapping broods in nature; the adults continue emerging from the infested plants without any definite interruption.

In order to get an idea of the rate of their emergence, five full-grown plants of *Amarantus caudatus* were caged in two separate wire-gauze cages and the adult weevils emerging therefrom were collected and recorded. It was noticed that the weekly number of adults emerging from the end of December to middle of January was somewhat higher than that emerging during the rest of the period, but there was no definite break in emergence during the experimental period.

The weevils are almost sexually mature after emergence, therefore the pre-copulation and pre-oviposition periods are short. Even in December, the weevils emerged on the 9th began to copulate on the 13th and started egg-laying on the 14th. The copulation lasts for about seven to eight hours and egg-laying may commence immediately thereafter.

Oviposition.—As stated already, the eggs and the method of oviposition were so far unknown. Fletcher [1917] surmised that the eggs are probably laid inside the stem under the axil of a leaf on one of the lateral branches. It has now been definitely observed that the female bores a hole, 1-1·5 mm. deep, in the tender branches of the plant or in the petiole or even midrib of leaves, and deposits a single egg therein (Plate XXVII, fig. 2). The mouth of the hole is closed with a sticky secretion spread into an oval flap. When fresh, this flap is dull green in colour and, therefore, it is difficult to make out the seat of oviposition without a careful examination. In a few days, the flap becomes black (Plate XXVII, fig. 1), but by this time the egg has already hatched and the grub bored into the stem. Older branches of plants, which are usually hard and somewhat dry, are avoided for oviposition.

During favourable season, *i.e.*, from April to September, the female lays two to three eggs per day (maximum six eggs) while during winter egg-laying may stop altogether. The maximum number laid by one female was found to be 174 spread over a life of about five months. The oviposition record of a typical female showed that during the first month following emergence, *viz.*, 9th December to 8th January, it laid twenty eggs. During the next month on account of severe cold, oviposition stopped altogether. It then laid sixty-four eggs during March and forty-nine eggs during April and died on 4th May after an oviposition period of about five months.

Egg.—The egg is oblong, broadly rounded at the two ends and measures on an average 1·2 mm. in length and 0·7 mm. in breadth (Plate XXVII, fig. 3). It has a uniformly pale yellow colouration and a smooth glistening surface. As it develops, it undergoes little change in shape or colour except that the length increases slighly at the expense of the breadth. Forty-eight hours after oviposition at 20°C., the dark reddish brown mandibles of the embryo become visible. They grow more and more prominent as hatching approaches and an hour before actual hatching they show a gnawing movement. As the eggs are laid in contact with cell sap of the plant, they require for their development not only a saturated atmosphere, but perhaps direct contact with free water. Thus the eggs removed from the plant and kept in a partially saturated atmosphere did not hatch at all. At room temperature, during March and November, the incubation of eggs takes ten to twelve days. Freshly laid eggs kept at constant temperatures of 27°C. and 20°C. hatched in about four days and ten days respectively.

Grub.—The freshly hatched grub (Plate XXVII, fig. 4) is smaller than the egg. It measures about 1·0 mm. long and 0·5 mm. broad. The head capsule is large, as broad as long, and constitutes about one third of the body length. It is light brown with several darker transverse streaks. The mandibles are

PLATE XXVII

FIG. 1. Dark spot showing the seat of oviposition by *Lixus truncatulus*

FIG. 2. Cross section of twig showing an egg of *Lixus truncatulus* inside the hole (×4)

FIG. 3. Egg of *Lixus truncatulus* taken out of the hole (×10)

FIG. 4. Grub of *Lixus truncatulus*, freshly hatched (×20)

FIG. 5. Pupa of *Lixus truncatulus*, (×6)

FIG. 6. Adult of *Lixus truncatulus*, (×8)

FIG. 7. Larva of *Lestodiplosis* sp. on weevil egg (×6)

FIG. 8. Adult of *Lestodiplosis* sp. (×20)

FIG. 9. Covering of hole cut open to show (*a*) Egg of *Pareuderus torymoides*, and (*b*) the weevil egg (×4)

FIG. 10. Adult of *Dinarmus sauteri* ♀ (×16)

FIG. 11. Adult of *Dinarmus sauteri* ♂ (×16)

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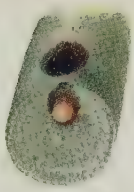

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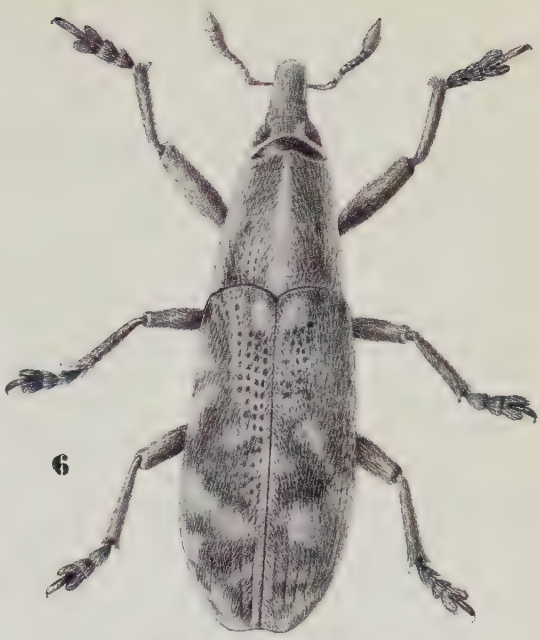

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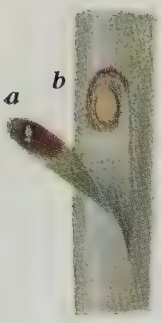

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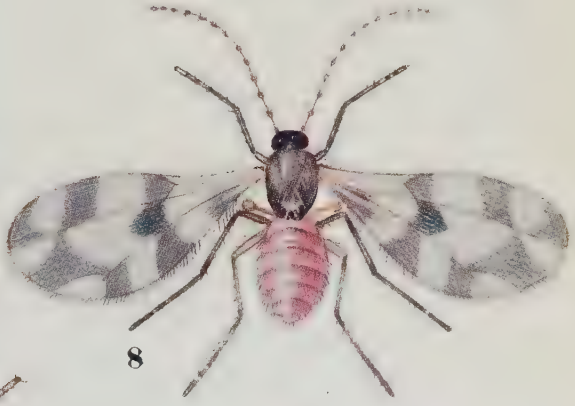

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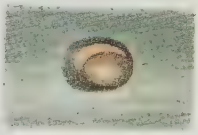

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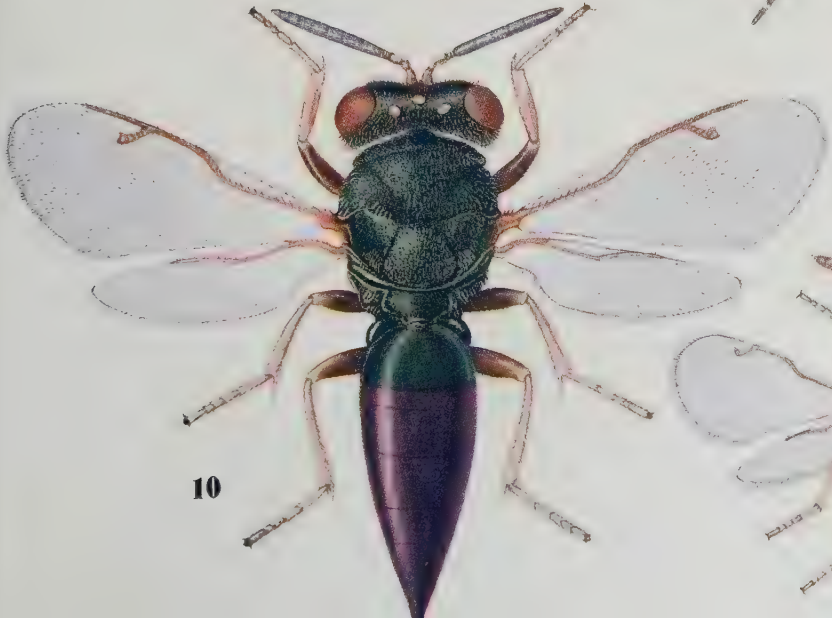


10

11

strong and dark brown in colour. The thorax and abdomen are uniformly light yellow. All the segments are nearly equal in breadth and size, excepting the last two abdominal segments which successively decrease in size. The terminal part of abdomen is thus broadly rounded dorsally.

The full-grown grub measures 14-15 mm. in length and 1·7 to 1·8 mm. in breadth. The description of full-grown grub as given by Gardner [1934] is reproduced below :—

" Body curved, slightly wider at thorax, posteriorly broadly rounded. Skin densely micro-asperate. Length about 15 mm. Head capsule reddish-testaceous with broad pale streaks, width about 1·75 mm. Frons wider than long, without dark median line. Ocelli one near antenna and a small posterior black spot on each side. Antenna with apical segment stoutly conical. Epipharynx with a row of six or seven long antero-lateral setae on each side, the stout rods contiguous posteriorly. Apical segments of palps small but distinctly longer than wide, labial palps separated by about twice width of basal segment. Typical abdominal terga with four distinct transverse folds, the fourth not protuberant. Spiracles nearly circular, with paired air-tubes projecting well beyond peritreme, posterior pair widely separated, subequal to preceding pair."

The grub stage of the weevil being a period of rather slow development, all attempts to complete this stage inside the constant-temperature incubators failed, as it was not possible to keep the growing plants in a healthy condition for sufficiently long period in the darkness of the incubator. On the other hand, the process of frequent transferring of the grub from old to fresh twig resulted, as in the case of many other borers, into extremely slackened development, often followed by death. Under conditions of room temperature, however, the grub stage, though very variable, was completed in several cases and the data are given in Table II. It will be noticed that grubs hatched about the end of March, pupated after forty to fifty-five days, while those which hatched in the end of October and beginning of November passed the entire winter in the larval stage.

TABLE II

Larval period of Lixus truncatulus *at room temperature*

Ref. No.	Date of hatching	Date of pupation	Pupal period (days)
17	30th Oct. 1935	Died on 15th Mar. 1936 without pupating	..
18	9th Nov. 1935	Died on 9th Feb. 1936 without pupating	..
19	26th Mar. 1936	20th May 1936	55
21	28th Mar. 1936	15th May 1936	48
24	5th Apl. 1936	24th May 1936	53
27	9th Apl. 1936	19th May 1936	40
27	9th Apl. 1936	24th May 1936	45
29	12th Apl. 1936	2nd June 1936	51

It is remarkable that the grubs can stand considerable starvation, particularly during the cold weather. A lot of fifteen well-developed grubs collected

from amarantus stem on 4th December 1935 was kept without food in the laboratory. Excepting two grubs which died during the first week, the rest died between 22nd February and 3rd March 1936. Thus the maximum period of starvation was as long as eighty-nine days.

Pupa.—When full grown, the grub prepares a wide chamber for pupation which causes a characteristic swelling of the stem or gall. The pupa (Plate XXVII, fig. 5) measures 8·7 to 9·0 mm. in length and about 2·6 mm. in maximum width. The eyes are dark from the very beginning, the rest of the body is pale yellow with dark brown lines at the inter-segmental areas of abdomen and antennae and along veins of the wing pads. The wing pads lie between the second and third pair of folded legs and extend a little beyond the tibio-femoral fold of third pair of legs. The abdomen is straight with prominent circular spiracles on the lateral aspect of segments.

Ordinarily the pupa lies motionless, but when disturbed, it moves its abdomen actively and can wriggle about a little. The duration of the pupal period as shown in Table III is nine to ten days at 27°C. as well as at room temperature during April, and twenty to twenty-four days at 20°C.

TABLE III

Pupal period of Lixus truncatulus *at different temperatures*

Ref. No.	Temperature	Date of pupation	Date of emergence of adults	Pupal period (days)	No. of weevils emerged
41	27°C. . .	9th Dec. 1935	18th December 1935	9	3
			19th December 1935	10	5
42	27°C. . .	17th Jan. 1936	26th January 1936 .	9	7
			27th January 1936 .	10	6
			28th January 1936 .	11	1
128	20°C. . .	1st Feb. 1936	21st February 1936.	20	2
			22nd February 1936	21	2
			24th February 1936	23	4
129	20°C. . .	3rd Feb. 1936	24th February 1936	21	1
			25th February 1936	22	5
			26th February 1936	23	3
			27th February 1936	24	2
136	Room temperature	5th Apl. 1936	15th April 1936 .	10	5
			16th April 1936	11	2
137	Do. .	9th Apl. 1936	18th April 1936	9	3
			19th April 1936 .	10	4
			20th April 1936 .	11	1

Adult weevil.—In general appearance the weevil (Plate XXVII, fig. 6) is dirty coloured with several dark patches on a densely pubescent body. It is rather large in size, measuring on the average 8·75 mm. long and 3 mm. broad. Though very sluggish and disinclined to fly or even run, it is quite alert in noticing an approaching hand. By a little but prompt movement it just turns round the stem away from the hand and gets concealed by falling on the ground and feigning death like many other Curculionidae. On falling down it lies ventral surface upwards and the legs stretched out. The ventral surface being uniformly dirty white matches exactly with the ground colour and thus seems to offer a successful protection against enemies.

When food is available the life of the adults is fairly long. A great majority of the weevils emerging in November and December lived up to the mouth of April and May next, thus obtaining a life of four to six months.

When kept without food the life of the weevils is considerably shortened. A number of freshly emerged weevils was starved at a constant temperature of 27°C. and two different saturation deficiencies. It was noticed that the average life of starved adults was 9·2 days (maximum 15 days) at a saturation deficiency of 9 mm. and 7·6 days (maximum 12 days) at a saturation deficiency of 0 mm. This shows that fully saturated atmosphere is detrimental to the longevity of adults, whereas it was essential for the proper development of eggs and grubs.

Number of broods.—From the foregoing account of the rate of development, it will be observed that under favourable conditions the total pre-imaginal period is roughly two months. This means that there are at least three broods from March to November. As already pointed out, owing to the rather irregular and variable larval development, the different broods in nature are not distinct. During winter all the stages of the weevil are found undergoing slow but some development during the day. Thus there is no true hibernation.

III. Parasites and predators of *Lixus truncatulus*

From the account of the life-history of *Lixus truncatulus* it will be observed that all its pre-imaginal stages are passed within the host plant; even the eggs are laid inside the plant tissue and covered over with a layer of sticky secretion. This would indicate that the weevil leads a well protected life from its enemies. Actually, however, it was found that it is prone to the attacks of a number of parasites, particularly in the egg stage. These are listed below :—

1. Egg parasites—
   (1) *Pareuderus torymoides* Ferriere
   (2) *Anastatus* sp. prox. *coleopterorum* Girault
   (3) *Anastatus* sp.
   (4) *Lestodiplosis* sp.
   (5) *Telenomus javensis* Dodd

2. Larval parasite—

(6) *Dinarmus sauteri* Masi

3. Pupal parasite—

(7) *Eurytoma curculionum* Mayr

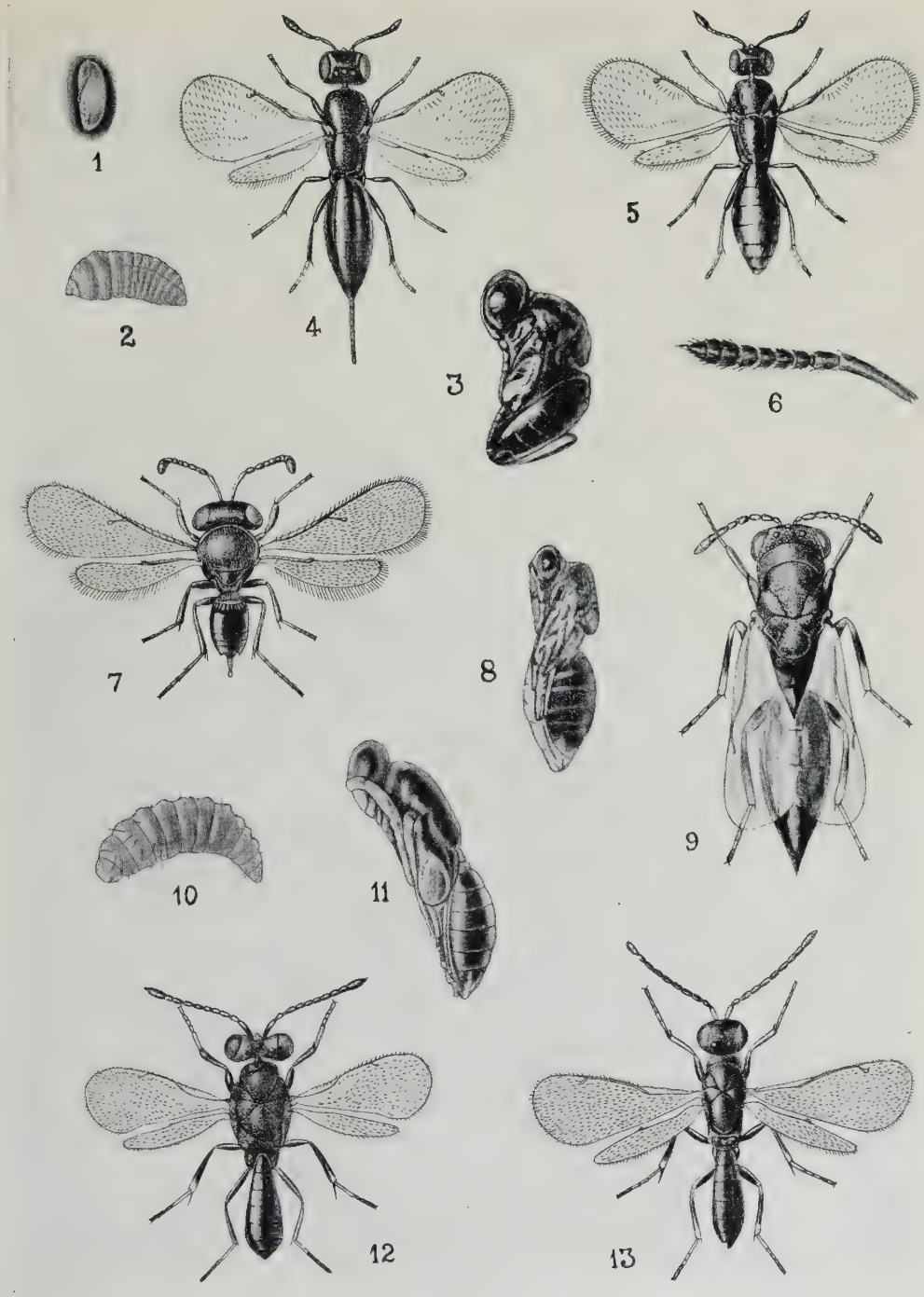
Recently Gardner (unpublished) has reared a species of *Calliephialtes* at Dehra Dun and Ayyar [1938] has bred some parasites of the genera *Tetrastichus*, *Dinarmus* (?) and *Spathius* from the immature stages of the weevil. Thus it is noteworthy that all the parasites dealt with in this paper have been bred from *Lixus truncatulus* for the first time, and were not known from Indian region so far. A detailed account of the life-history and morphology of the important ones is given in the following pages.

1. Pareuderus torymoides *Ferriere* (*Eulophidae* : *Chalcidoidea*)

Of the five egg parasites, listed above, *Pareuderus torymoides* Ferr. was found to be the commonest, and parasitised a larger proportion of eggs than those killed by all the other parasites put together. This is the first record of the species from India and perhaps the second in the world. It was only a few years ago [Ferriere, 1931] that the parasite was described from Java where it was found parasitising the eggs of *Alcides leeuwanii* infesting *Ceiba pentendra*. Beyond this first report of occurrence nothing is known of the life-history, status, etc., of the parasite. We have several species of *Alcides* injurious to vegetables in India and it is very likely that, as our knowledge advances, several additions will be made to the hosts of this apparently important parasite.

Systematic position and diagnostic characters.—This species is the genotype on which Ferriere [1931] erected the genus *Pareuderus* which is closely allied to *Euderus*. In order to facilitate the distinction of the species its important characters extracted from author's description are given below.—

" Head and thorax black with some bluish reflections or sometimes greenish on the thorax of males ; abdomen dark greenish or bluish violet on the sides. Antennae dark brown, pedicel with bluish shine, underside of scape yellow. Legs with bluish coxae and femora, the tibiae brown slightly bluish in the middle, the tip of tibiae and the tarsi white, except the last tarsal joint which is brown. Female (Plate XXVIII, fig. 4) : below the front ocellus a transverse furrow stretches almost from one eye to the other, separating the vertex from the frons. Face shagreened, slightly convex, the antennal groove not deep and without marginal carinae. Cheeks with length one third the length of an eye. Antennae inserted in the middle of the face, pedicel not broader than, and as long as, a third of the scape ; the four joints of the funicle subequal in length and as long as the pedicel ; club longer than the two preceding joints together, pointed at tip. Thorax entirely punctate, dull, the scutellum and the pleurae more finely punctate than the mesonotum, scutellum not higher than the mesonotum, but strongly convex on the sides and behind. Propodeon short, punctate, with a median carina and short furrows near the spiracles. Marginal vein longer than the submarginal, gradually and slightly narrowed toward the apex ; postmarginal vein thin, as long as one fourth of the marginal ; stigmal vein shorter than the postmarginal. Male (Plate XXVII, figs. 5 & 6): similar but the scutellum more convex at the sides and behind. Length : ♀ 1·5-1·6 mm. (without ovipositor) ; ♂ 1·2-1·4 mm.



FIGS. 1—6. *Pareuderus torymoides*— Fig. 1. Egg (×25), Fig. 2. Grub (×15), Fig. 3. Pupa (×25), Fig. 4. Adult, female (×25), Fig 5. Adult, male (×25), Fig 6. Antenna of male (×100)

FIG. 7. Adult ♂ of *Telenomus javensis* (×25)

FIGS. 8 & 9. *Eurytoma curculionum*—Fig. 8. Pupa (×8), Fig. 9. Adult (×12)

FIGS. 10—12. *Anastatus* sp.— ♂ Fig. 10. Grub (×15), Fig. 11. Pupa (×25), Fig. 12. Adult (×22)

FIG. 13. Adult of *Anastatus* sp. prox. *coleopterorum* ♂ (×25)

Life-history and description of various stages.

Oviposition.—The female parasite locates the egg cavity of the host, pierces the lid of the cavity with its ovipositor and deposits a single egg just above the host egg (Plate XXVII, fig. 9). It is interesting to note that the parasite is capable of discovering the host egg which is laid so securely inside the plant tissue.

The freshly laid egg (Plate XXVIII, fig. 1) is semi-transparent, becoming more opaque and whitish as development advances. It measures 0·36 mm. in length and 0·17 mm. in breadth. It is broadly rounded at the anterior end and somewhat narrowed posteriorly. At the broad end it carries a stalk, usually curved and about half as long as the egg. Due to its elastic shell the egg does not maintain symmetrical shape but under pressure one side may become more convex than the other.

At room temperature during December (17°-21°C.) egg hatches in about two days; at a constant temperature of 27°C. it hatches within twenty-four hours.

Grub.—Soon after hatching the grub goes underneath the host egg and begins to feed on the egg contents. Evidently the puncture made is so fine that egg contents do not ooze out immediately, and the egg retains its shape for a few days. Gradually the egg loses its turgidity, till it becomes empty and a well-developed parasite grub is seen under the host egg shell. One host egg is enough for the entire feeding stage of one parasite.

The well-developed grub (Plate XXVIII, fig. 2) measures about 1 mm. in length and 0·5 mm. in breadth. Body consists of a small head, three thoracic and ten abdominal segments. The first thoracic segment is the largest, the second and third are about half as broad as the first. The abdominal segments are much narrower than the thoracic segments so that the ten abdominal segments put together constitute only about half the entire length of the grub. The entire body of the grub is white with a faint bluish tinge. The sharp mandibles are, however, dark brownish.

The duration of the grub stage is rather short; it is five to six days at a constant temperature of 27°C. and about ten days at room temperature (17°-21°C.) during December.

Pupa.—The full-grown grub pupates within the cavity in which the host egg was laid. The pupa is stout, broad and very much curved (Plate XXVIII, fig. 3). The eyes are round and comparatively large and the antennae are rolled over the eyes reaching as far as the middle of abdomen. It measures about 1·25 mm. in length and 0·66 mm. in breadth.

The pupal period was determined from a large number of individuals, of which some typical cases are given below (Table IV) :—

TABLE IV

Pupal period of P. torymoides

Ref. No.	Date of pupation	Date of emergence	Pupal period (days)	Temperature (°C.)
43	13th December 1936 .	18th December 1936 .	5	27
44	14th December 1936 .	19th December 1936 .	5	27
54	20th December 1936 .	25th December 1936 .	5	27
61	21st December 1936 .	25-26th December 1936	5-6	27
70	25th December 1936 .	30th December 1936 .	5	27
74	25th December 1936 .	30-31st December 1936 .	5-6	27
90	4th January 1937 .	8th January 1937 .	5	27
		9th January 1937 . .	6	27
116	18th January 1937 .	24th January 1937 .	6	27
119	19th January 1937 .	24th January 1937 .	5	27
122	27th January 1937 .	4th February 1937 .	8	20

It will be noticed that the pupal duration is about five to six days at 27°C. and nearly eight days at 20°C.

Number of generations.—From the data given above we find that the pre-imaginal stages of the parasite are completed in less than a fortnight at 27°C. and in about three weeks during December (temperature 17°-21°C.). The host, on the other hand, is very slow developing; it takes over two months even under favourable conditions of temperature, *viz.*, 27°C. It has not been possible to study all the factors, but from the work carried out so far, it seems that the parasite has sufficient potential capacity to control the multiplication of the host. Under natural conditions during winter about 31 per cent of the weevil eggs were found parasitised.

2. Anastatus *sp. prox.* coleopterorum *Girault.*

3. Anastatus *sp.*

(*Encyrtidae* : *Chalcidoidea*)

The weevil egg is parasitised by two closely allied species of *Anastatus*, the life-history of which is somewhat similar to, but the incidence much lower than, that of *Pareuderus torymoides* F. Of these, *Anastatus* sp. prox. *coleopterorum* is comparatively more common than the other. Otherwise their life-histories are similar and are therefore dealt with together.

Oviposition.—Eggs are laid singly just under the lid of the cavity containing the host egg. The egg is translucent white, resembling in shape with, but slightly bigger than the egg of *P. torymoides*. It measures 0·44 mm. in length and 0·15 mm. in breadth. When about to hatch, the egg becomes slightly elongate.

Grub.—In about forty-eight hours the grub hatches out of the egg, goes under the host egg and begins to feed on it. After some time the host egg

loses its usual brilliance and turgidity and becomes dull and depressed. The parasite grub feeds so rapidly and voraciously for the first two to three days that by this time the host egg seems almost entirely consumed. Later, the feeding and development are slow, the larval period being completed in five to seven days at 27°C. (Table V). One egg is sufficient for the completion of the larval stage of the parasite. Superparasitism was noticed in one instance when a full-grown grub of *Anastatus* sp. was found along with a pupa of *Pareuderus torymoides* in a hole containing remnants of the host egg. The two parasites can probably develop together without detriment to each other and two individuals can complete development on a single egg of the host.

TABLE V

Larval and pupal periods of Anastatus *spp.*

Ref. No.	Temperature (°C.)	Date of hatching	Date of pupation	Larval period (days)	Date of emergence of adult	Pupal period (days)
64	27	22nd December 1935.	29th December 1935.	7	4th January 1936	6
81	27	31st December 1935	5th January 1936	5	11th January 1936	6
83	27	..	1st January 1936	..	8th January 1936	7
97	27	6th January 1936	12th January 1936	6	18th January 1936	6
99	27	..	7th January 1936	..	13th January 1936	6
100	27	9th January 1936	14th January 1936	5	20th January 1936	6
109	27	17th January 1936	22nd January 1936	5	29th January 1936	7
117	27	..	19th January 1936	..	26th January 1936	7
118	27	..	20th January 1936	..	27th January 1936	7
			Average .	5·6		6·4

The full-grown grub (Plate XXVIII, fig. 10) is elongate, measuring 1·5 mm. in length and 0·5 mm. in breadth. The head is large, glistening-white and turned downwards. The three thoracic segments bear a row of five to six long setae medially on either side of the body. The maximum thickness of the body is maintained up to the first five abdominal segments which are darker than the rest of the body owing to the food contents inside. The posterior segments of the abdomen gradually taper to the narrow anal and are smoky white in colour.

The grub leaves feeding a few hours before pupation which takes place within the plant cavity.

Pupa.—The pupa (Plate XXVIII, fig. 11) differs very markedly from that of *P. torymoides*, being more slender and longer as well as more erect than the latter. It is 1·5 mm. long and 0·5 mm. broad in the region of the thorax. The eyes are comparatively small, the antennae not reaching as far as the distal

end of folded wings and the legs reaching up to the tip of the abdomen. The abdominal segments are quite distinct.

The pupal period lasts six to seven days at a temperature of 27°C. (Table V).

Adult.—It will be desirable to give here briefly the distinguishing features of the two species of *Anastatus*.

Anastatus sp. prox. *coleopterorum* Girault ♂ (Plate XXVIII, fig. 13) : The adult is comparatively smaller, measuring 1·1-1·25 mm. long and about 0·25 mm. broad in the region of the thorax. Head and thorax are metallic green and the eyes are coppery red. Front femora are dark green while mid and hind femora are dark brown. The front tibiae and tarsi are brownish ; also mid and hind tibiae, except at apex, are brown. Basal three segments of tarsi are light brown, while the terminal two segments are darker. Axillae almost meet mesad. Marginal vein in front wings is somewhat equal to the submarginal vein. The abdomen is dark brick-red. Lateral ocelli are separated from the eye border by a distance equal to that between the front and the lateral ones.

Anastatus sp. ♂ (Plate XXVIII, fig. 12) : Length 1·25-1·50 mm., breadth about 0·5 mm. This species is readily separated from the above by its little larger size and uniform dark brown or black colour. Lateral ocelli are separated from eye border by a distance much less than that between the front and the lateral ones. Eyes are dark brown. The axillae are distinctly separate mesad. Legs except fore tibiae and tarsi and apices of mid and hind tibiae are concolourous with thorax. Except metatarsus, rest of the segments of mid and hind tarsi are dark. Thorax is black and relatively broader than in the other species. Marginal vein is somewhat shorter than the submarginal vein. Abdomen is dark brown.

Number of generations and incidence.—From Table V it will be observed that these parasites complete the pre-imaginal period, *i.e.*, from oviposition to emergence of adult, in less than two weeks at 27°C., the temperature obtaining during April-May at Pusa. In spite of the fact that they develop almost as fast as *P. torymoides* their incidence in nature remains very low throughout. This shows that other factors of environment are not suitable for their rapid multiplication.

4. Telenomus javensis *Dodd*

(*Scelionidae* : *Proctotrypoidea*)

A number of adults of the Scelionid parasite, *Telenomus javensis* Dodd (Plate XXVIII, fig. 7), emerged in cages containing *Amarantus caudatus* twigs infested with different stages of *Lixus truncatulus*. The exact status of the parasite and the stage of the host attacked are not known but previous records show that the whole group of Scelionidae comprises egg parasites without excep-

tion. It is, therefore, very likely that here too it attacks the egg stage of the weevil. The parasite is recorded from Java, where it was bred in 1914 from the eggs of a Tineid infesting sugarcane. Kieffer's [1926] revised description of the female of this species translated into English is given below :—

" Black. Head a little broader than the thorax. Eyes finely pubescent. Antennae 11-segmented, scape as long as the five following segments put together, length of second segment hardly twice its thickness, third segment much smaller but hardly thinner than the second, somewhat longer than thick, 4-6 segments almost similar, transverse. Club five segmented, its proximal segment short, proximal four segments transverse, the third being considerably thicker than the rest. Thorax just longer than broad, pubescent, and finely and irregularly sculptured. The fore wings reaching much beyond the tip of abdomen, hyaline, not very broad, marginal ciliation fairly long, surface hairs fine and thick, veins yellowish, the submarginal vein interrupted in the middle of the front margin, marginal vein half as long as the stigmal vein. Femur dark brown, tibia brownish yellow, tarsi yellow. Abdomen a little longer but not broader than the thorax, truncate apically, first tergite short, the second covering greater portion of the upper surface, first segment and the front part of second segment with longitudinal striation, the rest of the abdomen smooth and shiny. Length 0·9 mm."

5. Lestodiplosis *sp.*

(*Itonididæ* : *Diptera*)

During the intensive study of the life-history of *Lixus truncatulus* in which several hundreds of eggs collected from field were carefully examined, in four cases a cecidomyid larva was found in association with the weevil egg (Plate XXVII, fig. 7). Two of these larvae were successfully bred to the adult stage and were identified by Barnes as a species of *Lestodiplosis*. In none of the four cases under observation did the weevil egg hatch out and in one instance an apparently empty egg case of the weevil was noticed after the pupation of the cecidomyid larva. Due to insufficient material a more thorough examination of the relationship of the two insects was not possible. It is therefore difficult to state with certainty its exact status, but it seems very likely that the larva was predaceous on the weevil egg. Dr. Barnes' views on this point expressed in his letter dated 1st January 1937 are as follows :—

" It is not a predatory form and so probably came from *Amarantus*, not the *Lixus*. There are no previous records of gall midges of these genera being found on *Lixus* and *Amarantus*."

In view of the above remarks, it would appear to be a record of doubtful predatism ; but Barnes seems to have overlooked the fact that a species of *Lestodiplosis* is already known to parasitise the strawberry weevil, *Anthonomus signatus* in Minnesota [Marcovitch, 1917], while several species of *Lestodiplosis* are known to attack dipterous larvae and pupae including those of other cecidomyids [Kieffer, 1900].

The cecidomyid larva in the beginning of last stage measures 1·25 mm. long and 0·25 mm. broad. As in many other cecidomyids, the body is flat dorsoventrally, comparatively broad towards the posterior end, gradually tapering anteriorly, and bearing a pair of antennae. The three thoracic segments are as usual pink in colour and the sternal spatula situated ventrally on the first thoracic segment is rather small.

The pupa is about 1·25 mm. long and 0·5 mm. broad and possesses a general salmon-pink colouration. The abdomen is large and plump, the folded legs not going beyond the fifth abdominal segment. The head bears anteriorly a pair of spines, behind which are seen a pair of backwardly directed fine hairs. The thorax also carries a pair of spines.

The adult midge (Plate XXVII, fig. 8) is a little over 1·3 mm. long and 0·5 mm. broad. The antennae are long, thirteen segmented, each segment bearing a whorl of hairs at the base. The neck of all the segments is roughly of the same size and nearly three times as long as broad. Head is dark, the thorax pale brownish and the abdomen bright pink. The wings, which are 1·5 mm. long and a little over 0·6 mm. broad, are fringed with hairs. The third vein reaches just beyond the tip of the wing and fifth vein forked at about one third its distance from the tip.

6. Dinarmus sauteri *Masi*

(*Miscogasteridae* : *Chalcidoidea*)

This parasite was found parasitising to a small extent well-developed larvae of *Lixus truncatulus*. Masi [1926] described this species from specimens collected by Sauter from Formosa (Japan) in 1912. The writer has not been able to find any record of this species from any other part of the world since that date.

The grub is elongate and active. When full grown, it measures 2·7 to 2·8 mm. in length and 0·7 to 0·8 mm. in breadth. It pupates within the tunnel close to the remains of the host grub. The pupa is straight with rather small eyes, brownish antennae and a large abdomen. At 27°C. the pupal period is only four to five days.

Adult.—The original description of the species (op. cit.) is based on female specimens only. Masi has kindly sent me a revised translation of this which I reproduce below after slight modifications (Plate XXVII, fig. 10).

Female.—Length 4·5-5·0 mm. Aeneous-green; the abdomen above deep violet, except the basal tergite which is brassy-green; sometimes abdomen above violet-brown; eyes chestnut; antennae reddish yellow, ninth and tenth antennal segments (fourth and fifth of funicle) and sometimes the sixth (first of funicle) darker, also sometimes the 8th (third of funicle) testaceous and the following ones dark; the club always dark. Legs brownish, with ochraceous tarsi; knees, apices of mid tibiae, apical one third of posterior tibiae, whitish or yellowish, the darker colouration always sharply defined against the paler colour. Wings slightly greyish, veins dark brown.

Head shorter than long; antennae inserted above the ocular line in the middle of face; margin of the clypeus deeply incised at the middle; genal sulcus faint, orbits elliptical, vertex obtusely angulated and rounded. Scape reaching beyond the front ocellus; pedicel nearly as long as the three ring-joints combined; the third ring-joint one and a half times longer than the preceding one and a little broader; first funicular segment three times longer

than broad and twice the length of pedicel ; the following segments becoming gradually shorter and broader, the 5th a trifle longer than half of the 1st, one and half times longer than broad ; length of club equal to the first segment and the third ring-joint combined. Fore wings well rounded apically, ratio of the costal cell, marginal, post-marginal and stigmal veins 100 : 51 : 36 : 20 ; stigmal vein somewhat clavate, basal cell and mirror (naked area) naked, the remaining space covered with rather densely set setae ; of these at least 30 between the stigmal and post-marginal veins (on the upper side of the wing) four or five in each transverse series. Dorsellum (central part of the true metanotum) with the usual two pits at its extremities ; propodeum not carinated medially, densely punctate, with the posterior part distinctly bordered near the petiolar articulation ; stigma twice longer than broad, separated from the median area of propodeum with two superposed pits, the lower of which is more visible and deeper. ' Mesepimerum ' (morphologically inferior. part of mesepimerum) shining, seen with the microscope finely reticulate. Abdomen not longer than the rest of body, nearly equal to the thorax in breadth, very minutely reticulate.

As there is marked dimorphism in this species and the male has *not* been described so far, it has been considered necessary to figure both the sexes (Plate XXVIII, figs. 10 and 11) and to give below important points in which male differs from the female :—

Male.—Length about 2·4 mm. General colour bright metallic green. Excepting the apical third of first, the whole of the second, and the base of the third tergites which are white, the rest of abdomen very dark violet or dark coppery brown. Scape and pedicel light brown, rest of antennae dark brown. Coxae concolourous ; fore and mid femora reddish brown ; hind femora coppery brown ; basal halves of tibiae light brown ; all knees, apical halves of tibiae, tarsi except apical segment almost white. Veins relatively lighter than in the female. Antennae inserted well above the middle of face, ring-joints two, pedicel nearly equal to the first funicular segment. Propodeum with a distinct median carina, sculptured like the rest of thorax.

Allotype—1 male on a card, in the Pusa collection.

Paratype—1 male on a card, in the Pusa collection.

Pusa, T. Ahmad, ex grub of *Lixus truncatulus*, 9-12-1935.

7. Eurytoma curculionum *Mayr*

(*Eurytomidae* : *Chalcidoidea*)

The grubs of this parasite were found feeding on the grubs and pupae of *Lixus truncatulus* in small numbers. The parasite grub is a voracious feeder, measuring when full grown about 4 mm. long and 1-1·25 mm. broad. This species is known to parasitise grubs of *Cleopus campanulae* and *Gymnetron asellus* in Austria [Mayr, 1878], *Baris* spp. in France [Faure, 1923] and *Lixus junci* Boh., a pest of seed beet in central Italy [Menozzi, 1934 ; 1936].

In the latter case the parasite is considered to be of sufficient importance and attempts are being made to breed it artificially and liberate it for the control of *Lixus junci*.

The pupa (Plate XXVIII, fig. 8) has rather small, characteristically red eyes and brownish antennae on a small head. It measures 4 mm. long and 1·25 mm. broad. The thorax is prominently bulging out with distinct three segments. The folded legs do not go beyond three fourth the length of abdomen. The abdomen is plumpy, the length being only a little over one and a half times the breadth. The pupal period is eight to nine days at 27°C.

The adult parasite (Plate XXVIII, fig. 9) is a stout-looking black insect about 4 mm. long and 1·25 mm. broad. The entire body with the exception of eyes and legs are jet black. Eyes are deep brownish red. The tibiae and front tarsi are light brownish red. The femora are smoky black, while the middle and hind knees and tarsi are whitish excepting the terminal segments which are dark. The abdomen is elongate, about two and a half times as long as broad.

IV. Acknowledgments

I have great pleasure in recording my sincere thanks to Dr. H. S. Pruthi, Imperial Entomologist, for facilities and useful suggestions given during the work; to Mr. M. S. Mani, Assistant to Imperial Entomologist, for the identification of parasites in general and for the description of the male of *Dinarmus sauteri* in particular; to the Bureau of Entomology, United States Department of Agriculture for naming some parasites and to Mr. P. Narayanan, artist, for painting the plates illustrating the paper. Finally I must acknowledge gratefully the information furnished by the Entomologists, Madras, Dehra Dun and Indian Museum, Calcutta, regarding the distribution of *Lixus truncatulus* from the specimens of this species in their collections.

V. Summary

The Amarantus borer, *Lixus truncatulus*, is widely distributed throughout India, as a minor pest of several species of amarantus cultivated for vegetable, medicinal or ornamental purposes. The weevil has actually been bred from *Amarantus gangeticus*, *A. caudatus*, *A. viridis*, and *A. spinosus*. In case of scarcity of these food plants eggs are also laid after making several empty holes in *Chenopodium album*.

The adult weevils are almost mature on emergence. They copulate for seven to eight hours and begin egg-laying soon thereafter. Eggs are laid singly in holes bored in tender branches of plants, or in the petioles and midribs of leaves and covered over with a sticky secretion. The maximum number of eggs laid by a female during her life of about five months was 174. The incubation period is four days at 27°C. and ten days at 20°C. The grub stage lasts forty to fifty-five days during spring, while the grubs hatched in autumn

pass the entire winter in this stage. During winter the grubs can live without food for about three months. The pupal period is nine to ten days at 27°C. and twenty to twenty-four days at 20°C. The adult weevils normally live for four to six months. From March to November there are roughly three broods which overlap each other.

The life-history and morphology of five egg parasites, one larval parasite and one pupal parasite of the weevil are described. All these parasites have been bred from *Lixus truncatulus* for the first time from India. Of the egg parasites, *Pareuderus torymoides* Ferriere seems to be the most important ; it parasitises up to 31 per cent of eggs at Pusa. Two species of *Anastatus*, and *Telenomus javensis* Dodd come next and are of minor importance as egg parasites. A cecidomyid, *Lestodiplosis* sp., is also recorded as a predator on eggs of the weevil.

The chalcid parasite, *Dinarmus sauteri* Masi parasitises the grubs to a small extent. As there is marked dimorphism in this species and the original description is based on female specimens only, the male of the species is also described.

Lastly *Eurytoma curculeonum* Mayr is recorded as parasitising the grubs and pupae of the weevil in small numbers.

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THE BIONOMICS, LIFE-HISTORY AND CONTROL OF THE GRASSHOPPER *POECILOCERUS PICTUS* (FAB.)—A NEW PEST OF CULTIVATED CROPS IN NORTH INDIA

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(With Plates XXIX and XXX)

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I. Introduction

The grasshopper *Poecilocerus pictus* (Fab.) is quite familiar to the students of entomology and needs no special introduction. On account of its large size it is selected as a type for teaching insect morphology in some Agricultural Colleges. Though its anatomy has been studied in some detail, its bionomics, life-history, etc., have not been fully investigated. Obviously this is due to the fact that this grasshopper has not attracted the attention of economic entomologists since it is not considered to be a pest of any cultivated crop. It chiefly feeds on a wild plant called *ak* (Hindustani), a species of the genus *Calotropis*, and hence it is popularly called as the *ak* grasshopper. With

the exception of a record by Fletcher [1914] of this grasshopper having been found damaging young fig trees in South India, it was not known to cause damage to any plants of economic value until recently when in the summer of 1937 the writers noticed it causing considerable damage to brinjal, castor and tomato in a village garden at Shadipur near the estate of the Imperial Agricultural Research Institute. During April and May it was observed feeding on *ak*, but when it had almost exhausted this food plant it migrated to the neighbouring crops named above. It was thus realized that this grasshopper threatened to be a potential pest of cultivated crops and therefore it was considered advisable to study its biology, range of food plants, etc., and try some methods of control. The grasshopper has been closely studied during the past two years and the results of the investigations are reported in the following pages.

II. Distribution

This grasshopper is known to occur throughout the plains of India except in Bihar, Bengal and Assam. Outside India it is found in Africa. It appears that this grasshopper likes dry and hot climate.

III. Food plants and nature of damage

Poecilocerus pictus, as its popular name (*ak* grasshopper) indicates, is very fond of *ak* and feeds upon every part of it, *e.g.*, leaves, stems, pods, flowers, etc., often reducing the plants to naked stems, not even leaving the green bark behind. With a view to investigating the potentiality of this grasshopper as a pest of cultivated crops, experiments were carried out with numerous food plants available at Delhi during summer, when this insect is commonly met with in the field. In order to know whether the hoppers were capable of growing and developing on different food plants, they were confined in tall wire gauze cages with different kinds of leaves which were changed every morning. The following plants were tried :—brinjal, tomato, *bhindi* (*Hibiscus esculentus*), potato, gourds, cabbage, radish, musk-melon, castor, oleander, maize, cotton, broad bean, hollyhock, sorghum, cowpea, *pipal* (*Ficus religiosa*), tobacco and sugarcane.

Out of the plants named above, the hoppers fed readily on the leaves and fruits of brinjal and tomato, and on the leaves of *bhindi*, potato, gourds, cabbage, radish, musk-melons, castor, oleander and maize. They underwent normal moulting and became adults. The hoppers fed moderately on cotton, broad bean, hollyhock, sorghum and cowpea, and underwent moulting. The *pipal* leaves were only nibbled a little and sugarcane and tobacco leaves were not eaten at all.

In the field so long as *ak* plants were available, the grasshopper was mostly confined to them. Where brinjal plants were growing in the vicinity of *ak*, the hoppers were found infesting them but to a less extent. It appears that this insect becomes a menace to cultivated crops if its population becomes very large during spring when it is still on its wild food plant, *viz.*, *ak*.

IV. Life-history and description of immature stages

The life-history of *P. pictus* in broad outline is as follows :—

The eggs are laid in soil from June to August and sometimes even in September. The winter is generally passed in the egg stage and the young hoppers hatch out towards the end of March or beginning of April at Delhi. The nymphs feed and grow during April and May and become adults about the end of the latter month, after undergoing five or six moults. The males and females start copulating within one or two weeks of the final moult, and egg-laying and active breeding continues up to the end of September, by which time the last batch of eggs has been laid. After the middle of October hoppers begin to dwindle in numbers, but there are numerous egg masses in the soil during winter. Only a very insignificant percentage of the eggs laid in summer hatch in autumn but the great majority do not hatch till the ensuing spring, as stated above.

The life-history is described in detail below :—

The two sexes can be easily distinguished. The males are smaller and more slender than the females. The extremity of the abdomen in the male is conical and turned upwards, while in the female it ends in a number of paired chitinous hooks, which constitute the ovipositor.

Copulation

The male does not start copulating immediately after it becomes adult. The pre-copulation period in this sex varies from five to eight days. However, the female is ready for copulation soon after it undergoes the final moult. On several occasions fresh adult females were given to sexually mature males and the copulation started almost immediately. The process of copulation is almost the same as in other grasshoppers. It may last from two to four, sometimes even six hours and in rare cases even one day. The males as well as females were found to copulate several times, which may be as many as fifteen times within twenty to twenty-five days, and both polyandry and polygamy are quite common. There is frequent copulation both before and after oviposition.

Oviposition

The egg-laying begins from twenty to twenty six days after the emergence of the female as adult. The period intervening between the first copulation and the first egg-laying varies from fourteen to twenty days.

The ovaries of females of different ages were dissected to note the time of maturity of eggs. It was found that in the case of the females which had copulated, the eggs were mature after fifteen or sixteen days, while in the ovaries of the virgin females the eggs were found to be fully mature after twenty two to twenty four days. The pre-oviposition period in the virgin females was found to be twenty four to twenty seven days. Observations on the pre-oviposition periods and the number of ovipositions and eggs laid within a period of two months of adult life have been summarized in Table I.

TABLE I

Pre-oviposition period and number of ovipositions and number of eggs laid

Serial No.	Date of attaining adult stage	Date of first oviposition	Pre-oviposition period (days)	Date of subsequent oviposition	No. of egg masses laid	No. of eggs
1	11th June 1938	3rd July 1938 .	23	21st July 1938 .	2	Kept in soil to note hatching
2	13th „ .	5th „ .	23	22nd „ .	2	
3	14th „ .	18th „ . (found after some time).	35	...	1	120
4	15th „ .	4th „ .	20	...	1	40 (on the surface)
5	14th „ .	5th „ .	22	12th July 1938 .	2	...
6	14th „ .	5th „ .	22	...	1	110
7	12th „ .	...	...	18th July 1938 .	2	...
8	14th „ .	5th July 1938 .	22	...	1+1	108
9	14th „ .	5th „ .	22	12th July 1938 .	2	...
10	17th „ .	8th „ .	22	24th „ .	1+1	108
11	16th „ .	5th „ .	20	...	1	77
12	16th „ .	7th „ .	22	...	1	...
13	18th „ .	14th „ .	27	...	1	...
14	19th „ .	13th „ .	25	...	1	...
15	19th „ .	14th „ .	26	...	1	...
16	19th „ .	21st „ .	33	...	1	...
17	15th „ .	6th „ .	22	...	1	...
18	15th „ .	8th „ .	24	...	1	133
	15th „ .	9th „ .	25	...	1	120
20	17th „ .	6th „ .	20—21	...	1	117
21	17th „ .	8th „ .	22	...	1	105
22	16th „ .	7th Nov. 1938 .	22	...	1	...
23	16th „ .	5th July 1938 .	20	...	1	65
24	17th „ .	6th „ .	20	22nd July 1938 .	2	105
25	17th „ .	17th „ .	31	...	1	98 (on wood)
26	15th „ .	8th „ .	24	26th July 1938 .	2	...
27	14th „ .	5th „ .	22	21st „ .	2	...
28	11th „ .	4th „ .	24	13th „ .	3	...
				28th „ .	...	...
		Average .	23	...	1·4	116
			Virgin females.			
1	17th June 1938 .	12th July 1938 .	26	...	1	3 or 4
2	17th „ .	10th „ .	24	13th July 1938 .	2	...
3	17th „ .	11th „ .	25	...	1	102
4	29th „ .	22nd „ .	24	...	1	...
5	19th „ .	17th „ .	29	...	1	...
6	17th „ .	...	...	...	1	140
		Average .	25 days	...	...	...

7
8
9
6
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11
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3a
4
12
13
1
2
14
15
16

PLATE XXIX

FIGS. 1 & 2. *Poecilocerus pictus*, females, in the process of egg laying. Note that the abdomen has been extended upto the length of about 5 in. (Fig. 1). In Fig. 2. the abdomen is being retracted after eggs have been laid, ($\times\frac{1}{2}$)

FIG. 3. Freshly laid egg ($\times 2\frac{1}{2}$), with a part of the chorion highly magnified

FIG. 3*a*. The egg about to hatch ($\times 2\frac{1}{2}$), with a part of the chorion highly magnified

FIG. 4. Freshly hatched young hoppers. Natural size

FIG. 5. Young 'larva' ($\times 2\frac{1}{2}$)

FIG. 6. 1st instar nymph ($\times 2\frac{1}{2}$)

FIGS. 7—12. Antenna and compound eye of the I—VI instar nymphs

I	$\times 10$
II—III	$\times 8$
IV	$\times 5$
V—VI	$\times 3$

FIG. 13. Lateral view of thorax of the 1st instar nymph ($\times 10$)

FIG. 14. Lateral view of thorax of the 3rd instar nymph ($\times 8$)

FIG. 15. Lateral view of thorax of the 5th instar nymph ($\times 5$)

FIG. 16. Sixth instar nymph ($\times 1\frac{1}{2}$)

The eggs are laid in the soil about 5-6 in. below the surface. Generally the soil which is selected for this purpose is of loose texture, hence more easily penetrable. The gravid females have inflated abdomens. When ready for egg-laying, the female trails its abdomen on the ground searching for a suitable spot by scratching the surface of the soil with its ovipositor. When such a spot has been found it starts making an egg chamber with the help of the anterior and dorsal valves of the ovipositor. The valves act like the prongs of a forceps. The egg-laying process has been studied in the laboratory in a large glass cage (1-5 ft. × 1 ft. × 1 ft.) which was half filled with soil. The top of the soil was covered with a thick card board leaving the soil uncovered near the sides only. The female was thus forced to bore holes in close proximity to the glass sides and thus the whole process could be easily watched. During the course of boring, the extremity of the abdomen rotates through an angle of 180°, pressing and making compact all sides of the burrow. The formation of the egg chamber takes from one to two hours. The chambers are generally 6-6½ in. deep. Though the abdomen is only 1·4 in. long in the normal position, it can be extended to reach the depth of chamber with the help of the elastic intersegmental membranes (Plate XXIX, fig. 1). In many cases the female makes several chambers and discards them without laying any eggs therein probably because of the unsuitable texture or moisture content of the soil. Sometimes the female turns its abdomen in a zigzag fashion if it meets with any obstacle inside the egg chamber. Before the eggs are actually laid the female exudes a fluid which is worked up into a frothy mass by the quick motions of the ovipositor valves and is deposited at the bottom of the egg chamber about 2 in. thick on which the eggs are laid one by one in a spiral manner so as to form a compact mass (Plate XXIX, fig. 2). The female gradually retracts its abdomen as the chamber gets filled up with eggs. When all the eggs have been laid, the vacant space in the chamber is filled with froth up to the top. The whole process takes about two to six and a half hours.

The female looks somewhat sluggish for a short time after oviposition. Generally one or two and sometimes three egg masses were laid by a single female during its life time under laboratory conditions. The unfertilized females generally laid one egg mass only. The number of eggs in a mass varied between 60 and 140, the average being about 116. It is likely that more than two egg masses are laid in nature.

Egg

The egg (Plate XXIX, fig. 3) is elongated, curved, almost cylindrical, thick in the middle and tapering towards the ends, which are bluntly rounded. When freshly laid, the egg is yellow but soon turns brown. The micropyle is darker in colour and can be easily demarked. When mature, the egg grows in thickness (Plate XXIX, fig. 3 *a*). There are two egg coverings, the chorion and the vitelline membrane. The former is sculptured and in a mature egg can be peeled off easily by scratching. The vitelline membrane is yellow, smooth but tough and cannot be pierced when pressed with an ordinary needle. When the egg is about to hatch, the young one inside can be seen through the

egg skin, moving its abdomen and striped hind femora. Just prior to hatching some black spots make their appearance on the surface of the chorion near the operculum. From these spots some transparent liquid oozes out, and it is at these spots that the membranes rupture and the 'vermiform larva' hatches out. Its head and antennæ (Plate XXIX, fig. 5) are bent downwards and the legs are folded. These "larvæ" make their way up through the dry froth by wriggling motions of their bodies and emerge out of the burrow in large numbers and present a very interesting appearance (Plate XXIX, fig. 4). The emergence of all the 'larvæ' from a single egg mass takes about ten minutes. Soon after reaching the surface of the soil, the 'larvæ' start the process of casting their covers, known as the intermediate moult. Just before this moulting, the cervical ampulla gets inflated and presents a two-knobbed swelling. By the alternate swelling and contraction of this ampulla the thin white skin is ruptured dorsally in the thoracic region and eventually the young nymph crawls out. The process of the intermediate moulting is completed within two to four minutes. Some previous authors have not considered this moult to be true one, but we, like Uvarov [1928], do not find any important difference between this and the subsequent moults.

The newly emerged nymphs are negatively geotropic, always trying to move upwards and ascending on any piece of stick or twig. They move in a mass towards the source of light and sit in clusters on any standing twig with their heads pointing upward (Plate XXIX, fig. 4). The nymphs begin to feed about twenty four hours after their emergence.

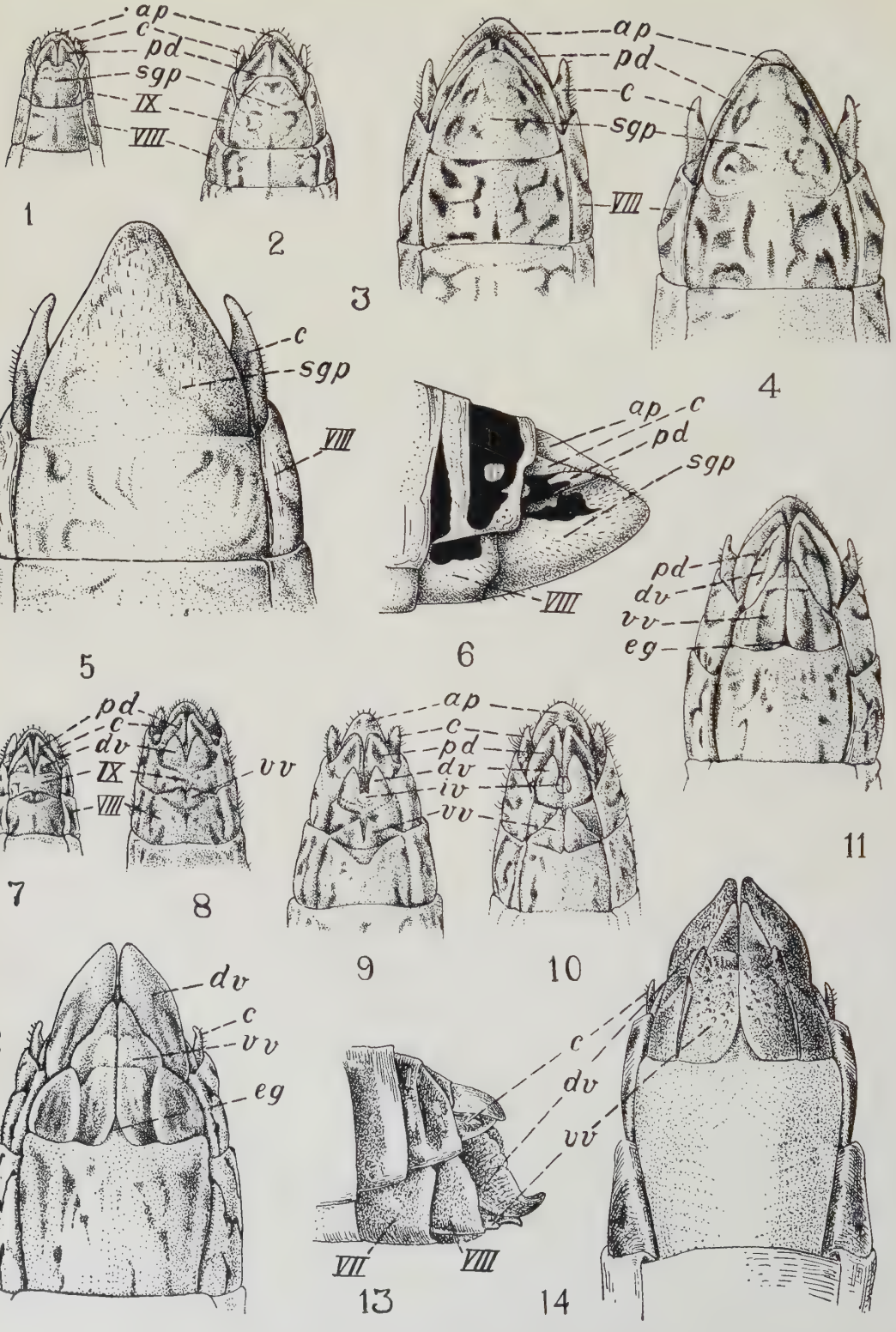
There are usually six nymphal moults before the individual reaches the adult stage. Sometimes there are five moults only. The duration of the various instars is variable. On an average (200 observations) they are as follows during summer :—

1st instar	9·7	days.
2nd „	7·8	„
3rd „	7·8	„
4th „	8·5	„
5th „	9·9	„
6th „	11·6	„

The total nymphal life lasts for fifty to fifty-six days during summer. In the case of those few eggs which hatch in autumn, the nymphal period is very long. Only a few of these nymphs become adults in November while the great majority of them over-winter and become adults in the following April after a nymphal life lasting seven or eight months.

Different Nymphal stages

First stage nymph.—The freshly emerged nymphs (Plate XXIX, fig. 6) are orange coloured, with white and faint blackish stripes. After the lapse of one day the blackish patterns become dark and the hoppers look yellowish grey in general appearance. They measure 9-12 mm. in length and about 2·5 mm. in breadth (in the region of the thorax). The pronotum is about 1·8 mm. long and the antenna about 2·25 mm.


ap
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VIII
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vv
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VII

PLATE XXX

FIG. 1—4 Posterior abdominal segments of the male nymphs of 1st, 3rd, 5th, and 6th instars respectively ; ventral view ; ×10

FIG. 5 Posterior abdominal segments of the male adult grasshopper ; ventral view ; ×10

FIG. 6 The same in lateral view ; ×7

FIGS. 7—12 Posterior abdominal segments of the female nymphs of 1st to 6th instars respectively ; ventral view ; ×10

FIGS. 13—14 Posterior abdominal segments of the adult female grasshopper ; lateral and ventral views respectively ; ×7

LEGEND TO LETTERING

ap—anal plate ; *c*—cerci ; *dv*—dorsal valves of the evipositor ; *eg*—egg guide ; *iv*—inner valves of the ovipositor ; *pd*—podical plates ; *sgp*—subgenital plate of the male ; *vv*—ventral valves of the ovipositor ; VII-VIII—seventh and eighth abdominal segments.

The head is conical and sloping in the front view. It projects forward between the compound eyes and forms a triangular area known as the fastigium. The margins of the fastigium are slightly raised. The eyes are oval, black and devoid of any stripe. The frons is traversed by a deeply sulcate frontal ridge, running from the tip of the fastigium up to the clypeus. The margins of the ridge—the frontal carina—are whitish in colour, and are bordered on each side by two yellow or orange patches.

The antennæ (Plate XXIX, fig. 7) are deep purple and are invariably eightsegmented. The first or the basal joint is cylindrical and the second is small and globular. The third to seventh segments are almost subequal. The eighth segment is very long and is equal in length to the three preceding segments taken together.

The pronotum is saddle shaped and has a very feeble medial carina. There are five yellow patches on each lateral lobe, the arrangement of which is constant as shown in the figure (Plate XXIX, fig. 13). The prosternum bears a very acute compressed aciculate tubercle, which is situated between the two coxæ. The mesonotum is a small narrow sclerite partly hidden below the pronotum. The metanotum resembles mesonotum except that it is broader than the latter. The metapleuræ have each a long oblique orange patch, in front of which are the small, almost circular spiracles (*sp.*) which are externally provided with two-lobed valves. The first pair of spiracles remain hidden under the pronotum.

The first and second pair of legs are small and have black and white markings all over. The third pair is stout. The upper and lower ridges of the hind femora are traversed with eight to nine and five to six black dots respectively. The hind tarsi are three jointed, the terminal joint being provided with a pair of claws and a median pad, the arolium.

The abdomen is almost cylindrical. It has a mid-dorsal stripe of white spots interrupted with black markings. On each side of this stripe there is a lateral interrupted stripe of orange spots. Ventrally the abdomen is silver grey, with black lines interspersed all over. In both sexes ten terga are distinct, the last two being partially fused. The eleventh tergum and the supra-anal plate are fused into one piece at the sides of which and slightly lower in level are the two small cerci, the appendages of the eleventh abdominal segment. The number of apparent sterna in the two sexes are different. In the male there are ten sterna including the first one which is fused with the sternum of the metathorax. The ninth sternum is large, slightly depressed ventrally and deeply notched at the posterior margin ; this is the rudiment of the subgenital plate (Plate XXX, fig. 1, *sgp*). Above this plate there is a pair of triangular outgrowths known as the podical plates (*pd*). In the female also ten sterna are visible, the ninth being cleft into two triangular pieces, which are the rudiments of the dorsal valves of the ovipositor (Plate XXX, fig. 6, *dv*). Lying above the pieces there is a pair of triangular podical plates (*pd*) as in the male. At the posterior margin of the eighth sternum there is a very tiny pair of protruberances which are the rudiments of the ventral valves of the ovipositor (*vv*)

It is not easy to distinguish sexes at this stage as the rudimentary subgenital plate of the male which is notched at the posterior margin, and the ninth sternum of the female which is cleft into two parts, looks very much like. But the presence of the rudimentary ventral valves of the ovipositor, resent at the posterior margin of the eighth sternum, distinguishes the emale from the male (Plate XXX, *cf.* figs. 1 and 7).

Second stage nymph.—The second stage nymph resembles in general appearance and colour the first stage nymph, but is bigger in size, measuring 10-13·5 mm. in length and 2·5 mm. across the pronotum. The antennæ are 3·0 mm. long and are usually nine-jointed (Plate XXIX, fig. 8), the sixth joint of the first stage having divided into two. Sometimes the antennæ consist of eight segments only. On the distal margin of the third to sixth joints there are two small white spots which are totally absent in the first stage. The dark compound eyes develop each a transverse whitish stripe.

There is no indication of the wing pads in this stage but the posterior angles of the meso and meta-nota become somewhat extended outwards getting thinner and slightly dark in colour.

There is no change in the abdomen except in the appearance of some new yellow patches, the broadening of the black patterns, and the further development of the external genitalia.

The ninth sternum or the subgenital plate of the male grows in size and the notch begins to flatten out. In the females the ventral valves and dorsal valves of the ovipositor grow longer and gradually extend backwards and cover part of the ninth segment and anterior half of the podical plates respectively (Plate XXX, fig. 8).

In this stage spiracles are visible on the lateral folds of the first eight abdominal segments, the first pair of spiracle being near the tympanum.

Third stage nymph.—The general colour of the body becomes deeper in this stage. The median yellow spots on the dorsal side of the abdomen turn orange in colour. The length of the body is 15-17 mm.

The antennæ (Plate XXIX, fig. 9) in this stage are about 4 mm. long and are ten or eleven jointed, the fifth joint and sometimes sixth as well of the previous stage having each been divided into two segments. The tenth or the last segment is dull purple in the basal half and light purple in the distal region, and can therefore be mistaken for two joints.

The pronotum measures 2·7 mm. The meso and meta-nota develop distinct, thin triangular outgrowths at their postero-lateral angles. These outgrowths are the rudiments of the future wings (Plate XXIX, fig. 14, *wp.*).

The subgenital plate of the male completely loses its notched appearance and becomes rounded at the posterior margin (Plate XXX, fig. 2). In the female the eighth segment develops a crescent shaped fold (*eg*) directed inwards near the bases of the ventral valves which now cover the base of the dorsal valves. This fold is the rudiment of the egg guide which becomes distinct in the fifth stage. A small transverse pad-like growth (*iv*) appears in this stage also between the bases of the dorsal valves (Plate XXX, fig. 9).

Fourth stage nymph.—The general colouration becomes still deeper in this stage. The body measures 20-24 mm. long and 3-4 mm. broad. The eyes are light brown near the lateral carina of the front, and their transverse stripes become broader than in the previous instar. There are tv elve to fourteen joints in the antennæ which are now 5·6 mm. long (Plate XXIX, fig. 10). The third and fourth segments show an incomplete division, while the fifth segment of the previous instar has divided into two segments.

The pronotum measures 3·2 mm. in length. The postero-lateral angles of the meso-notum and meta-notum grow thin and dark and acquire oblique folds and thus form the rudiments of wing pads (Plate XXIX, fig. 15). Further, in some nymphs towards the end of this stage the wing pads begin to turn upwards, and the mesonotal pair comes to lie below the metanotal pair. However, in great majority of the nymphs the wing pads turn in the fifth stage.

In the male, the subgenital plate becomes conical and spatula-like in appearance. The podical plates get gradually pushed up and become compressed due to the development of the subgenital plate. In the female (Plate XXX, fig. 10) the ventral valves extend further backwards and their posterior ends become narrow and pointed. The dorsal valves also grow bigger, the transverse pad at their base develops a longitudinal fissure resulting in two minute appendages (*iv*), which are the rudiments of the inner pair of valves of the ovipositor.

Fifth stage nymph.—The general colouration of the body develops a shining yellow tinge in the places where there were yellow and white patches in the previous stages. The orange coloured spots grow deeper in tinge. The size of the hopper increases, being about 25-27 mm. in length and 4·5-5 mm. in breadth. The antennæ (Plate XXIX, fig. 11) are fifteen-jointed and measure 7-8 mm. The third to tenth segments are laterally compressed and rhomboid in cross section ; those from eleven to fifteen are more or less cylindrical.

The anterior margin of the pronotum remains straight as in the previous stages but the posterior margin becomes convex at the middle. The wing pads get upturned in this stage, if they had not done so in the previous stage, and develop numerous unbranched veins. In the male, the subgenital plate reaches the supra anal plate (Plate XXX, fig. 3). In the female the ventral valves grow thicker and extend very much backwards, reaching the middle of the dorsal valves. The latter also grow and extend up to the tips of the podical plates. The egg guide (*eg*) is bigger and distinct (Plate XXX, fig. 11).

Sixth stage nymph.—The freshly moulted nymph of this stage (Plate XXIX, fig. 16) is lemon yellow in general colour with black markings. All the white patches of the previous stage become yellow and the black patches grow deeper in tinge. It measures about 35-43 mm. in length and 7·5 mm. in breadth. The description of this instar relates to those specimens which turn their wing pads in the fifth instar. It is noteworthy that the upturned state of wing-pads last in every case for two stages only, that is to say, the nymphs which turn their wings in the fourth stage become adult at the end of

the fifth stage after undergoing five moults only, and those which turn their wing-pads in the fifth stage become adult at the end of the sixth stage. The latter condition is more common than the former.

In this stage the eyes turn slightly bluish in colour and their transverse stripes disappear altogether. The antennæ measure 11-13·5 mm. and have fifteen or sixteen joints. The posterior margin of the pronotum grows backwards and covers the bases of the wing-pads, which in this stage are larger, thicker and dark blue in colour.

In the male, the subgenital plate becomes a conical, pouch-like organ into which the ædeagus is lodged. This plate extends beyond the extremities of the podical plates, hiding them from the ventral view, the margins of the supra anal plate being only visible (Plate XXX, fig. 4). In the female, the egg guide becomes fully developed and remains pushed in between the two ventral valves. The dorsal valves grow very thick and extend far beyond the podical plates. They develop a lateral fold externally. The ventral valves develop a transverse fold in the basal region and a vertical fold in each of the lateral lobes and thus appear to be made of several pieces (Plate XXX, fig. 11).

Just before the nymph is about to moult finally for attaining the adult stage its colour and different parts of the body undergo very well marked changes which can be easily made out through the old skin. All the black patterns on the body begin to turn bluish and all the orange and yellow spots fade away and instead continuous yellow patches on the blue background begin to appear. The basal region of the antennæ and the frontal ridge become bluish. The anterior half of the pronotum gets constricted, whereas the posterior half becomes broadened and blue in colour. The second pair of wing-pads begin to show a reddish tinge.

The ventral and dorsal valves of the ovipositor become highly chitinous. The tips of the latter curve upwards and their upper surface becomes broadly dentate. Each ventral valve develops a lateral tooth-like process in posterior region and a number of minute spines in the basal region. The lateral lobes of the ventral valves become very large and appear as distinct sclerities (Plate XXX, figs. 12 and 12-A.).

The final moult.—The process of final moulting is very interesting . The hopper, when about to moult, stops feeding and rests on a twig. It begins to contract and expand the anterior region of its body. The head is moved forwards and backwards and the pronotum is moved up and down by contraction of the cervical ampulla, causing the rupture of the skin along the medial line above the pronotum. This fissure gradually increases extending from the fastigium up to the wing-pads. The head and thorax begin to be gradually drawn out of the skin through the slit. The upturned wing-pads become horizontal in position and are drawn out of their sacs. The first and second pair of legs are then gradually drawn out. The third pair of leg is the last to come out. The cast skin is gradually left behind as the adult grasshopper draws itself out. During the whole process of moulting the hopper breaths hard and

keeps on expanding and contracting its body. Just after the moult the fore-wings are only partly expanded and the hind pair is folded like a fan. Gradually the wings are fluttered and raised vertically and within twenty to twenty-five minutes the elytra (fore wings) become fully expanded and develop bluish green colour with yellow veins and reticulations. The hind wings are hyaline to begin with but gradually develop a pink colour at the apex, which ultimately deepens into a red rosy colour. The insect then rests on the twig generally facing sunlight. A few minutes after, it starts feeding.

V. Control measures

Sprays

With a view to finding out some suitable insecticide for controlling this pest a number of stomach poisons were tried in the form of sprays, dusts and poison baits. Lead arsenate which is commonly used for such insects was not found effective either in the field or in the laboratory when sprayed at the usual concentration of 2 lbs. per 100 gallons of water. It did not kill any hoppers within ten to thirteen days. Between thirteen and sixteen days the mortality was 25 to 40 per cent. The hoppers were kept under observation in this and the following experiments for about sixteen days only because it was considered that if the poison would not kill the hoppers within a fortnight it would not have any practical utility.

In the control cage all hoppers were found living beyond sixteen days. The lead arsenite spray was prepared by dissolving 5 lbs. of stone-lime in 100 gallons of water to which 2 lbs. of powdered lead arsenate and 10 lbs. of *gur* were added and the whole mixture shaken thoroughly.

Sodium arsenite.—Sodium arsenite, which is a poison soluble in water, was found more effective. It was sprayed in laboratory cages at the rates of 2 lbs., 1 lb., and $\frac{1}{2}$ lb. per 100 gallons of water. With the dose of 2 lbs., 96 per cent of the hoppers were killed in eleven days, death starting within two days of feeding, and the average longevity period being six days. This dose was however found to be injurious to the sprayed plants and hoppers did not feed readily on them. With the dose of 1 lb., the mortality was 100 per cent in thirteen days, the death starting within three days of feeding and the average life being 8·5 days. This dose also proved harmful to the leaves and the hoppers did not feed well on the sprayed food. With the dose of $\frac{1}{2}$ lb. per 100 gallons, the leaves were not at all injured, but the rate of mortality was rather slow, being 90 per cent in twenty days, death starting within five days of feeding, and the average life being thirteen days. The hoppers fed moderately on the treated food. In the control cages all hoppers were alive after twenty days.

The above doses were repeated in the field on brinjal, castor, *jowar*, cucurbits, etc. The doses of 2 lbs. and 1 lb. per 100 gallons proved injurious to the leaves which moreover became unpalatable to the hoppers. The latter were present on the sprayed plants in less numbers on the next day. On the third or fourth day the treated plants were entirely devoid of hoppers

but a search for the dead ones did not reveal any carcases. With a dose of ½ lb per 100 gallons, there was no leaf injury. The hoppers were found feeding on the treated plants for a few days but gradually declined in numbers. However, the dead hoppers could not be traced easily, only one or two stray dead specimens being found under the plants here and there.

The sodium arsenite spray was prepared by dissolving 5 lbs. of lime and 1¼ lbs. of *gur* in 100 gallons of water and adding 2 lbs. of the poison. The spray was diluted twice or four times respectively when one lb. or ½ lb. doses were tried.

Calcium arsenite.—Calcium arsenite being insoluble in water does not affect the leaf tissue like sodium arsenite. It was used at the strengths of 2 lbs. and 1 lb. per 100 gallons of water. Both the strengths proved harmless to the leaves of *Calotropis*, cotton and oleander.

With a dose of 2 lbs., 95 per cent of the hoppers, feeding daily on the poisoned food, died in seven days in the laboratory cages, death starting within three days of spraying and the average life being five days. The hoppers however did not feed well on the sprayed food. Under field conditions 60 per cent of the hoppers feeding on the sprayed plants died within nine days, death starting within four days of feeding and the average life being 5·5 days. These hoppers had been allowed to remain on the sprayed plants in the field for thirty-six hours and then removed and put in the laboratory cages containing untreated food. The hoppers in the laboratory experiments were fed on sprayed food every day.

With a dose of 1 lb., the kill was 90 per cent in six days in the laboratory, the death starting within three days of feeding and the average life being four days.

Oleander plants were sprayed in the field with the above doses. The results were quite encouraging. The hoppers which had fed for forty hours on the sprayed plants, were removed and caged with sprayed food and about 90 per cent of them died in five days.

Dusting experiments

Sodium arsenite.—*Calotropis* and castor plants were dusted in the field with sodium arsenite mixed with lime in the proportion 1 : 4. The hoppers were collected after twenty-four hours of dusting and caged. From the treated plants only 33 per cent of hoppers died in six days, and the rest continued to live beyond sixteen days. They started dying on the fourth day after dusting. The leaves of *Calotropis* seemed more or less unaffected but those of castor plant showed a scorching effect.

Calcium arsenite.—Calcium arsenite dusted in the same proportion showed slightly better results. The leaves were not injured and about 50 per cent of hoppers died in five days. The remaining lived beyond sixteen days.

Paris green.—Paris green was mixed with fine wood ashes in the proportion of 1 : 8 and 1 : 16, and dusted over the *Calotropis* and oleander plants. Hoppers were allowed to feed for twenty-four hours on the dusted plant.

With a dose of 1 : 8, 60 per cent of the hoppers died in ten days, the remaining living beyond sixteen days. Hoppers started dying on the third day after dusting.

Sodium fluosilicate and lime in the proportion of 1 : 4 were similarly dusted on brinjal plants but very few hoppers were killed.

Baits

Sodium fluosilicate.—This bait was prepared in the usual way. One lb. of finely powdered sodium fluosilicate was mixed thoroughly with 20 lbs. of wheat bran ; 1 lb. of *gur* or 1 pint of molasses was dissolved in a small quantity of water and added to the bran mixture so as to make it into a crumbly mass. Some amyl acetate was added to give the bait a good smell of ripe banana fruits. The bait was scattered in the hopper-infested field but did not prove effective. The hoppers, which, as already described, seldom leave the plant and come down on the soil, did not take to the bait scattered on the ground. The hoppers kept in cages in the laboratory also did not feed on the bait.

Conclusion

Experiments described above show that the spraying of infested plants with calcium or sodium arsenite at the rate of 1 lb. and $\frac{1}{2}$ lb. per 100 gallons of water respectively seems to be the most satisfactory method of control.

VI. Summary

The grasshopper *Poecilocerus pictus* has been known to feed mostly on *ak* plant (*Calotropis* sp.), which is hardly of any economic value, but in the summer of 1937 it was found attacking brinjal and castor in a village garden near the estate of the Imperial Agricultural Research Institute.

Numerous food plants of economic value were tested in 1937 and 1938 to determine the potentialities of this new pest of cultivated crops. It is found that this grasshopper readily feeds on brinjal (leaves and fruits), tomato, castor, *bhindi*, oleandar, etc.

The seasonal-and life-histories and general bionomics of the grasshopper have been studied. The hoppers undergo five or six moults before becoming adult. The various instars have been described.

Several stomach poisons have been tested as sprays, dusts and baits. Spraying with sodium arsenite at the rate of half pound per 100 gallons of water has proved to be the most satisfactory method of control. Sodium fluosilicate bait, which is usually effective against surface grasshoppers, has not been found satisfactory due to the peculiar habits of the grasshopper.

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A NOTE ON THE DETERMINATION OF NITRATES IN WATERS AND SEWAGE

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Introduction

During the course of the analysis of sewage and effluents from the Activated Sludge Plant, Central Farm, Coimbatore, considerable difficulty was experienced in the determination of nitrates, especially in presence of large amounts of nitrites and soluble organic matter. The phenoldisulphonic acid method of determining nitrates in waters is the one most used and has been found reliable and quick, but owing to the presence of appreciable amounts of nitrites in the effluents, it was considered necessary to oxidise the nitrites beforehand either by potassium permanganate ($KMnO_4$) or by hydrogen peroxide (H_2O_2) as recommended in the standard methods [1925] of water analysis. In certain preliminary experiments where potassium permanganate was used as the oxidising agent considerable loss of nitrate was found to occur ranging from 35 to 90 per cent. This was rather surprising and a detailed study of the method was made to find out the causes of such loss.

Experimental

Experiment 1.

To trace the defects, if any, in the technique and procedure adopted for the determination of nitrates an experiment was conducted using dilute potassium permanganate for the oxidation of the effluents under different conditions and treatments.

Treatment.—(1) Neutralisation of the clarified effluent with dilute sulphuric acid ($N/10$) before adding neutral potassium permanganate and removal of the chlorides by silver sulphate;

(2) Oxidation of the clarified sample with acid potassium permanganate till the pink colour persisted, followed by neutralisation with $N/10$ sulphuric acid and removal of chlorides by silver sulphate;

(3) Addition of neutral potassium permanganate to the raw sample before neutralisation, clarification and removal of chlorides;

(4) Oxidation of the raw sample with acid potassium permanganate and determination of the total nitrates by Harper's method [1924].

The above experiment under each treatment was carried out in duplicate avoiding excess of silver sulphate in all the cases and the results obtained are recorded in Table I. It is surprising to note from the table that in all the treatments, the total nitrate values obtained are quite erratic and are far below the actual nitrites present in the samples.

TABLE I

Nitrite—N and total N (nitrite and nitrate) in the samples under different treatments

Treatment No.	Nitrite—N (parts per 100,000)	Average of two estimations		Per cent decrease of total N over nitrite—N
		Total N (NO_3+NO_2) after oxidation (parts per 100,000)	Decrease of total N over nitrite N (parts per 100,000)	
		Sample I		
1	0·150	0·010	0·140	93·33
2	0·150	0·008	0·142	94·66
3	0·150	0·015	0·135	90·00
4	0·150	0·026	0·124	82·66
		Sample II		
1	0·0187	0·0090	0·0097	51·87
2	0·0187	0·0072	0·0115	61·50
3	0·0187	0·0120	0·0067	35·83
4	0·0187	0·0110	0·0077	41·18

Experiment 2.

To confirm further the inapplicability of potassium permanganate, neutral or acidic, for the oxidation of small amounts of nitrites present in waters, the following experiment with pure sodium nitrite solutions was carried out.

Treatment.—(1) Ten c. c. of sodium nitrite solution (0·05 mg. N) made up to 50 c. c. and then dilute neutral potassium permanganate added at the laboratory temperature (27—28°C.) till the pink colour persisted and nitrate determined as usual after evaporation to dryness ;

(2) Ten c. c. of dilute (sodium nitrite 0·05 mg. N) solution treated with neutral potassium permanganate at 50·C. and nitrate estimated as in (1) ;

(3) Same as (2) but for the addition of potassium permanganate at 100°C ;

(4) Ten c. c. of dilute sodium nitrite (0.05 mg. N) solution treated with acidified potassium permanganate at the laboratory temperature (27—28°C.) and nitrate estimated as in (1) ;

(5) As in (4) but for the oxidation of the nitrite by acid potassium permanganate at 50°C ;

(6) Similar to (4) but for the oxidation of the nitrite at 100°C.

The experiment was duplicated for all treatments and the averagere sults obtained in each case are recorded in Table II.

TABLE II

Nitrite—N added and recovered as nitrate—N under different treatments

Treatment No.	Nitrite—N added (mg.)	Nitrite—N recovered as nitrate—N after oxidation with $KMnO_4$, average of two estimations	Per cent recovery of nitrite—N as nitrate—N
1	0·05	0·0225	45·00
2	,,	0·0247	49·40
3	,,	0·0252	50·04
4	,,	trace	*nil*
5	,,	,,	,,
6	,,	,,	,,

The results show that the recovery of the added nitrogen varies from 45 to 50 per cent where neutral potassium permanganate was employed for oxidation, irrespective of temperature considerations, and practically nothing in cases where acidified potassium permanganate was used.

Experiment 3

As an alternative to the potassium permanganate oxidation, hydrogen peroxide (Merck's) containing twelve volumes of oxygen was tried with varying quantities of sodium nitrite (A. R.) solution, 1 c. c. of which is equivalent to 0·005 mg. N before proceeding to sewage proper. The nitrite solutions were diluted to 50 c. c. each and treated with 5 c. c. of hydrogen peroxide added in small quantities of 0·5 c. c. followed by repeated boiling. A control was also run with hydrogen peroxide to make allowance for any nitrate that might be present in it. The results recorded in Table III show that practically all the added nitrites have been recovered. The hydrogen peroxide was tested and found free from nitrate.

TABLE III

Nitrite—N added and the nitrate—N recovered after oxidation with H_2O_2

No. of dilutions	Nitrite—N added (mg.)	Nitrate—N after oxidation with H_2O_2, average of two estimations (mg.)	Per cent recovery of the nitrite—N as nitrate—N after oxidation with H_2O_2
1	0·025	0·0246	98·4
2	0·040	0·0398	99·5
3	0·050	0·0493	98·6
4	0·075	0·0752	100·27

Experiment 4

The method was next extended to sewage proper adopting the following treatments and the results compared with those obtained by Harper's [1924] method.

Treatment.—(1) Oxidation of the clarified effluent after neutralisation and removal of chlorides, with successive additions of hydrogen peroxide in small lots of 0·5 c. c. each (the total quantity of hydrogen peroxide may vary from 2 to 5 c. c.) followed by repeated boiling ;

(2) Same as (1) without clarification ;

(3) Determination of nitrates in the sample according to Harper's [1924] method.

Blanks were run with a known quantity of hydrogen peroxide and nitrite in each case determined in a separate aliquot of the filtrate. The results recorded in Table IV for the total nitrates after oxidation are quite promising, being at any rate, unlike in the potassium permanganate method, greater than the actual nitrites in the sample. The free nitrates estimated by Harper's [1924] method in the original sample were, however, far below those obtained by the oxidation method even after making allowance for the nitrite in the sample.

TABLE IV

Nitrate—N in the effluent, obtained by the H_2O_2 oxidation process and Harper's method

Treatment	Nitrate—N, average of 4 estimations (parts per 100,000)	Nitrate—N total, after oxidation with H_2O_2, average of 4 determinations (parts per 100,000)	Free nitrate—N in the sample by difference (parts per 100,000)
1	0·038	0·252	0·214
2	0·038	0·253	0·215
3 Direct determination of Nitrate—N by Harper's method .	..	..	0·123

Experiment 5

In this experiment a known amount of nitrate (0·05 mg. of nitrite—N) was added to a definite volume of effluent from the Sludge plant after clarification and removal of chlorides. The recovery of the nitrite nitrogen added was effected almost completely as nitrate nitrogen after oxidation with hydrogen peroxide free from nitrate. A control was also run with the effluent under identical conditions to make allowance for the nitrite and nitrate nitrogen present therein. As the sample in question was of very low nitrite content, less than one part per million, nitrates were also estimated directly both in the control and the sample to which known amounts of nitrite were added according to the standard method [1925] without preliminary oxidation. From the results of the experiment recorded in Table V, it is clear that the nitrate nitrogen obtained without oxidation is very much below that of the oxidised sample, even after deducting the little nitrite nitrogen present therein. It would appear therefore from this experiment that the oxidation of the clarified effluents by hydrogen peroxide is quite essential even when the nitrites present therein are very low.

TABLE V

Nitrate recovery in effluent by the oxidation and direct processes

Sample	Nitrate—N added (mg.)	Nitrite—N in the sample (mg.)	Total N after oxidation, average of four estimations (mg.)	Nitrate—N oxidation method (mg.)	Nitrate—N by the direct method, average of four estimations (mg.)	Per cent loss of nitrate—N, direct method over the oxidation procedure
Effluent alone . . .	*Nil*	0·0076	0·0325	0·0249	0·0125	—50·20
Effluent + sodium nitrate .	0·05	0·0076	0·0820	0·0744	0·0293	—39·39
Recovery of added N by the direct and oxidation methods by difference .	...	...	...	0·0495	0·0168	...

The following procedure may be finally recommended for estimation of nitrates in polluted and other waters.

One hundred to two hundred c. c. of effluent or water are treated with enough freshly precipitated aluminium hydroxide (10 c. c. for every 100 c. c. will be sufficient) followed by the addition of a calculated quantity of silver sulphate solution (8 grms. per litre) to remove the chlorides present in the sample. The mixture is stirred well and filtered. A convenient aliquot from the filtrate corresponding to 50—100 c. c. of the original sample, according to its nitrate content, is neutralised very nearly with dilute sulphuric acid using methyl orange as indicator and then treated with nitrate free hydrogen peroxide (about 3 —5 c. c. for every 50 c. c. have been found sufficient) in small quantities of 0·5 c. c. each followed by repeated boiling for a few minutes. The sample after oxidation is evaporated to dryness in a porcelain dish on a water bath and the residue treated and stirred well with 2 c. c. of Phenol-disulphonic acid. After allowing the mixture to stand for ten minutes it is taken up with 15—20 c. c. of distilled water and transferred to a Nessler cylinder. Then 1 : 1 ammonia is added till the yellow colour appears and the final volume made up to the 100 c. c. mark, after the mixture has cooled down to the laboratory temperature. The liquid is stirred well and the nitrate nitrogen determined by comparison with freshly prepared nitrate standards.

DISCUSSION

The use of acid potassium permanganate for oxidation of nitrate solutions is common and under greatly dilute conditions of this reaction oxidation takes place quantitatively. But the utter disappointment met with when this reagent was used for the determination of small quantities of nitrates present in waters and effluents by the phenoldisulphonic acid method may be due to the following causes :—

(1) It is a recognised fact that when a nitrite solution is treated with standard potassium permanganate in acid medium, it is necessary to use a very dilute solution containing at least 5,000 parts of water for one of nitric

acid to avoid decomposition of the nitrate by sulphuric acid. When the nitrite solution after oxidation with acid potassium permanganate is evaporated to dryness, the concentration of the residual acid, if any, will increase and decompose the nitrate formed in the beginning. This appears more probable in view of the blank results recorded (Table II) in cases where acid potassium permanganate was resorted to for the oxidation of nitrites.

With neutral potassium permanganate as the oxidising agent, the reasons for the loss of nitrate are much less clear. When hydrogen peroxide is used as an oxidising agent, it has been found that the recovery of added nitrite nitrogen to effluent as nitrate nitrogen was almost complete. It is also evident from the results recorded in Table IV that the nitrate nitrogen obtained without oxidation for a sample of very low nitrite content, less than one part per million, is very much below that of the oxidised sample, even after making allowance for the nitrite nitrogen present therein. This low value might be due to the partial reduction of the nitrates by the soluble organic matter present in the clarified effluent during evaporation to dryness. The observation incidentally gives a clue to the probable role, the soluble colourless organic matter and other reducing substances in a clarified sample would play in the determination of nitrates. It looks as though it is absolutely essential, whether there are appreciable quantities of nitrites or not, to oxidise the reducing substances that may be present in clarified effluents or waters free from chlorides, for correct estimations of nitrates therein.

Summary

For a correct estimation of nitrates in sewage and other polluted waters the preliminary oxidation of nitrites, organic matter and other reducing substances in the clarified samples is imperative and for this purpose hydrogen peroxide has been found more effective and reliable than potassium permanganate.

Details of the technique adopted have been outlined.

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SOME CITRUS NURSERY TECHNIQUE TRIALS AT THE FRUIT RESEARCH STATION, ANANTARAJUPET, MADRAS PRESIDENCY

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(With Plate XXXI)

Introduction

The method of bud-insertion in citrus trees is fairly standardised in this country. It consists of the removal of bud-shield, which carries with it a small portion of the wood of the bud-stick at the time of primary slicing out, but which is carefully removed before insertion of the bud-shield within the " T " or the inverted " T " shaped slit made on the seedling rootstocks. On the other hand, in America [Camp, 1931 ; Coit, 1930 ; Hume, 1926 ; and Wickson, 1926] and in the West Indies [Commission Agriculture, 1905] the general practice at present is to insert the bud-shield with a piece of wood attached to it. Even in the case of other fruits, the American writers advise the retention of a slice of wood in the bud-shield [Bailey, 1903, 1920 ; Chandler, 1928 ; Knapp, 1929 ; and Stahl, 1925]. In South Africa, it has been pointed out that the retention or otherwise of the slice of wood in the bud-shield is largely a method of individual preference or experience [Powell, 1930]. In England, Baltet [1910] has also advocated the retention of a thin slice of wood in the bud-shield, while removal of wood is favoured in some other English publications [Hall and Crane, 1933 and Ministry of Agriculture, 1935]. Work on apples in England has shown that the American practice of retaining the slice of wood in the inserted bud-shield has not established the superiority over the other method, although it has been pointed out that the results are required to be confirmed by further trials during different seasons [Garner, 1934]. The American method has also been reported to have afforded no material benefit in the trials conducted in Palestine. Writers in Australia are divided in their opinion with regard to the advantage or otherwise of retaining a slice of wood [Anon, 1932 and Cock, 1937].

In regard to the optimum period when the seedling rootstocks of citrus are to be lopped after bud-insertion, there is also some divergence of opinion prevailing among citrus nurserymen in most parts of the world. It appears

that in this country, most of the nurserymen lop off the citrus rootstocks either immediately after bud-insertion or within about five to six weeks afterwards or after the bud-sprouts have put forth two to six inches of new growth. Bailey [1903 and 1920] suggests that in outdoor conditions the rootstocks should not be cut back to the bud in one operation but in two ; the first one, soon after the stocks show signs of growth activity when the stock is cut back to a stub over the bud-joint, and second, about two weeks later after the bud has begun to grow, when the stock is cut off to bud-joint. Almost similar recommendations are made by Powell [1930], who further states that, if the stock is cut off close to the bud in the first instance, it might die back and cause the death of the bud. Davies [1917] in South Africa recommends cutting back of the stock to three or four inches above the bud as soon as these are about to shoot out.

The present investigations have been undertaken with a view to ascertain the desirability or otherwise of retention of the slice of wood in the orange bud-shield and the optimum period for carrying out the primary lopping of the rootstocks. The most suitable period for budding oranges in this Station was also intended to be ascertained.

Material and methods

The trial was conducted during the seasons 1936-37 and 1937-38. In the former year, scion was obtained from a single tree of Kodur *chinee* orange, *C. sinensis* Osbeck, and the buds were inserted on eighty seedlings from the same parent of Guntur sour orange, *C. aurantium* Linn. *var.* Hort., every month and during a period of four successive months, commencing from June 1936. One operator was responsible for all the 320 bud-insertions. All the 320 seedlings were growing together in a compact plot of the same age and of apparently uniform vigour. The age of the seedling rootstocks at the time of the first bud-insertion on 9th June 1936 was about twelve months.

The following four treatments were included under study and twenty seedling rootstocks in each of the four months were budded or lopped off according to each of the treatments :—

Treatment No. 1.—Bud inserted with a thin slice of wood adhering to it, and stock seedling lopped off to about three inches above the bud after the bud-sprout has made extension growth of not less than two inches in length.

Treatment No. 2.—Bud insertion after removing the wood from the bud-shield, and stock seedling lopped as in Treatment No. 1.

Treatment No. 3.—Bud inserted as in Treatment No. 1, and stock seedling lopped off to three inches above the bud immediately after bud-insertion.

Treatment No. 4.—Bud inserted as in Treatment No. 2 and stock lopped as in Treatment No. 3.

The second lopping of the rootstocks up to the bud in all the four treatments was done only at the time of lifting the budded plants from nursery beds. In every series, records of the percentage "take" of buds, and the days taken for "bud-break" were collected and recorded. Exactly four months after the date of bud-insertion, the height measurements of bud-sprouts were also collected in every case.

The results obtained during 1936-37 were so striking and interesting that it was considered necessary to lay out a more comprehensive trial in the following year. This consisted of a series of three separate trials with the same treatments as described below :—

(*a*) Seedling rootstocks from one *kichili* (*C. maderaspatana* Tanaka) parent treated by four different methods as explained in 1936 trials, with the scion obtained from a single *chinee* orange (*C. sinensis* Osbeck) parent. The bud-insertions were done during the last week of July 1937 by one operator.

(*b*) Seedling rootstocks from a second *kichili* (*C. maderaspatana* Tanaka) parent treated as above, using scion from a second *chinee* orange (*C. sinensis* Osbeck) parent. The bud-insertions were done from 7th to 11th August 1937 by a second operator.

(*c*) Seedling rootstocks raised from the second *kichili* (*C. maderaspatana* Tanaka) parent referred to under (*b*) above and treated similar to (*a*) and (*b*) using scion from a single acid lime (*C. aurantifolia* Swingle) parent. The buds were inserted from 16th to 20th August 1937 by the same operator mentioned under (*b*).

The seeds of all the rootstocks used in the three aforementioned trials had been sown on the 7th November 1935 and the primary transplantation from seed to nursery beds had been done on the 15th September 1936. All the seeds had been sown on raised seed-beds of made up soil in a small area in a compact plot. The transplantation of the seedlings reserved for each of the three trials had been done to three separate but contiguous plots. Roughly 600 seedlings of apparently uniform size had been planted to nursery beds for each of the three trials.

During the first week of July 1937, *i.e.*, almost immediately before the insertion of orange buds, measurements of stem thickness of individual seedlings growing in the plot reserved for the first of these three trials were collected. The analysis of these measurements showed that the mean of 352 plants was 0·83 cm. with a standard deviation of 0·21, and the coefficient of variability was therefore 25·3 per cent. In order to show a 10 per cent difference between the two means it would require eighty-four observations for a level of $P = 0{\cdot}01$ [Hoblyn, 1931 and Vaidyanathan, 1936]. Neither the area nor the number of plants available rendered the selection of such a large number of individuals possible, having regard to the necessity of providing an adequate number of guard rows. Furthermore, it was expected on the basis of the results of 1936 that the differences between the treatments would be

of fairly large proportions. The fact that measurements collected related to only the seedling rootstocks, whereas the ultimate data are to be collected on scion worked on these rootstocks, renders it also not absolutely necessary to base the number of replications strictly on growth measurements of the root-stocks.

It was, therefore, decided to have four repetitions of rows, containing a minimum number of sixteen buddable seedlings, thus involving in all a minimum of sixty-four buddable seedlings under each treatment in each of the three trials.

The number of treatments randomised in each block was four, and this was identical to the four treatments described above.

The following was the plan of layout of trials designated as (*a*) on page 653.

Guard rows

Guard rows	Block I	Block II	Block III	Block IV	Guard rows
	1	3	3	1	
	2	1	2	3	
	3	4	1	2	
	4	2	4	4	

Guard rows

A similar plan of layout was adopted in the other two trials also, with a separate randomisation of plots in the last case.

The total number of seedlings budded by each of the four treatments in each block in trial (*a*) is given below :—

Blocks	Treatment No. 1	Treatment No. 2	Treatment No. 3	Treatment No. 4	Total
I . . .	16	16	16	16	64
II . . .	16	17	16	16	65
III . . .	19	17	16	19	71
IV . . .	16	17	18	20	71

In order to ascertain the optimum season for budding sweet oranges to seedling rootstocks, a separate investigation was planned out in 1936 independently of the abovementioned trials. Two citrus varieties—*kichili* (*C. maderaspatana* Tanaka) and *gajanimma* (*C. pennivisiculata* Tanaka) which are grown fairly extensively in this tract—were selected for these trials. The scion was selected from a single *chinee* orange tree, and the bud-insertions were made by one operator every month from June 1936 to May 1937. From June to September 1936, the number of rootstocks of each of these varieties budded every month varied from ten to seventeen according to the availability of seedlings in a compact plot and uniformity of vigour. During the remaining months buds were inserted on to twenty seedlings of each of these rootstocks. The age of the rootstocks, however, could not be kept constant during every bud-insertion owing to the practical difficulties involved in securing seeds for sowing every month. The budding technique employed in the experiment was the one described under method No. 1 above.

Results of 1936-37 trials

The data collected during 1936 are summarised and presented in Table I.

Table I

"Bud-take", "Bud-break" and growth of the bud-sprouts, four months after bud-insertion

Stock—Guntur sour orange *Scion—*Chinee *orange*

Date of bud insertion	Method No. 1			Method No. 2			Method No. 3			Method No. 4		
	"Take"	"Bud-break" period in days	Height	"Take"	"Bud-break" period in days	Height	"Take"	"Bud-break" period in days	Height	"Take"	"Bud-break" period in days	Height
	Per cent		(cm.)	Per cent		(cm.)	Per cent		(cm.)	Per cent		(cm.)
9-6-1936 . .	67·0	30 to 40	16·4±1·85	42·0	30 to 40	14·5±1·57	56·0	10 to 15	10·6±1·15	25·0	10 to 15	11·5±1·59
7-7-1936 . .	100·0	Do.	16·0±1·67	83·0	Do.	14·0±1·21	75·0	Do.	10·0±2·20	71·0	Do.	8·0±1·14
11-8-1936 . .	83·0	Do.	12·5±1·17	62·0	Do.	9·0±0·59	61·0	Do.	5·7±0·62	50·0	Do.	2·1±0·38
7-9-1936 . .	100·0	Do.	18·4±2·06	65·0	Do.	13·2±0·89	70·0	Do.	11·5±1·93	40·0	Do.	11·7±2·31

The following interesting facts appear to have emerged from this investigation :—

(*i*) The number of plants produced from buds with wood attached to the bud-shield is consistently greater than the number produced from those inserted after removal of wood on identically lopped rootstocks. The difference is so large and the similarity of results under different seasons so strikingly great that, the superiority of inserting the bud with the wood over the common Indian method of inserting the bud after removal of wood may be said to be clearly established.

(*ii*) " Bud-break " appears to be clearly stimulated by lopping of the stocks at the time of bud-insertion. A difference of three weeks is noticeable in favour of lopped stocks in all the four months.

(*iii*) The height measurements of the bud-sprouts collected four months after bud-insertion also reveal consistently significant differences. In spite of an earlier " bud-break " on lopped rootstocks, it will be noticed that the amount of growth in terms of length of new shoots on the lopped stocks is markedly less than on those stocks which were lopped one month after insertion. The data, therefore, indicate that the budded plants will be available for planting within a shorter period of time if the lopping operation is delayed for a month.

(*iv*) The presence of wood in the bud-shield does not appear to be an important factor in determining the growth processes on the rootstocks, as the lopping treatment.

(*v*) Both in the matter of " bud-take " and subsequent growth of bud-sprouts, interesting indications of the seasonal effects have been afforded by these data. July has proved to be the optimum month for budding operations, followed by September, while in June, the " bud-take " has been uniformly poor under all the four treatments.

(*vi*) The rate of growth of bud-sprouts has been comparatively more rapid in the September batch. June and July may also be classed as favourable months in this respect, while August has proved to be responsible for a comparatively poor rate of growth on this rootstock.

It seems safe to infer from these data that July and September are the optimum months for the propagation of *chinee* orange by budding in this tract, and that the presence of a slice of wood in the bud-shield and delayed lopping of the rootstocks till the bud-sprouts have made a sufficient growth of not less than two inches in length, are conducive for producing not only a large number of plants but also an earlier maturation of bud-sprouts.

Results of the trial on 'the optimum season for budding' carried out during 1936-37

The results of the separate investigation carried out to ascertain the optimum season for budding of oranges are presented in Table II.

TABLE II

Percentage "bud-take" of chinee *orange on the rootstock varieties, 1936-37*

Months	Stocks		Mean
	Kichili	*Gajanimma*	
June	77	90	83·5
July	100	100	100·0
August	88	82	85·0
September	95	100	97·5
October	55	60	57·5
November	20	60	40·0
December	45	75	60·0
January	90	90	90·0
February	90	90	90·0
March	20	25	22·5
April	45	50	47·5
May	30	45	37·5
Mean	62·9	72·3	

There is a fairly wide difference in the percentage "take" of orange buds between the two rootstock varieties in favour of *gajanimma*, which produces about 9 per cent more "bud-take" in the case of the twelve months, while the variations due to several months are also highly marked.

On the basis of the above results, the months are arranged below in the descending order of magnitude in percentage "take":—July, September, January, February, August, June, December, October, April, November, May, March.

The optimum months for successful budding were, therefore, July and September. During the remaining months high " take " of buds were obtained during January, February, August and June while April, November, May and March were found to be decidedly the most unfavourable.

July and September, therefore, have proved to be the most favourable months for budding oranges on three rootstock varieties in two independent trials.

From a reference to the rainfall and temperature records during 1936-37, it appears that the months which have proved to be the most unfavourable for budding operations have been such that have either been characterised by heavy precipitations or prolonged hot and dry spells. For instance, the heaviest rainfall recorded during the year was in November, while the maximum temperature under shade had reached above 100°F. only during March, April and May. It appears possible, therefore, that extremes in wet weather or temperature are both important limiting factors in the propagation of citrus by means of budding.

It has also been observed that excessive dry and hot weather prevailing between March to June has considerably injured the young sprouts. A few sprouts emerging from the February insertions also were damaged by the hot weather prevailing in the following months.

The suitability of February and possibly of the latter half of January for the operation is, therefore, open to question in spite of the high percentage of success obtained during these months. Considering all these points, it seems that July to September provides the optimum period for budding oranges in this tract, but the suitability of any of these months may be influenced by the wet weather conditions prevailing during the bud-insertion period. The latter point seems to be of importance, in that, while the month of August produced less success during 1936 than July and September, during 1937 it was found that August produced the maximum percentage of success in all the series of bud-insertions totalling about 2,000 on about six different rootstocks, while July to September, though continued to produce high " take ", were much behind August in this matter.

Results of 1937-38 trials

Trial (a)

All percentages have been analysed after the Sin^{-1} transformation and ' No. of days ' after the square root transformation [Cochran, 1938]. For splitting into single D. F., Yate's [1934] approximate method has been used where there were one or two ' missing ' values in some classes.

" *Bud-take* ".—The mean percentage " take " and the analysis of variance worked out for the percentage " take " obtained in the 1937 trials are presented in Tables III and IV.

TABLE III

Mean percentages "take" (X)—*Nursery Technique Trials*

Stock—Kichili. *Scion*—Chinee *orange.*

Serial No.	Blocks	Treatment No. 1	Treatment No. 2	Treatment No. 3	Treatment No. 4	Total	Mean
1	I	68·75	56·25	93·75	56·25	275·00	68·75
2	II	88·24	75·00	87·50	68·75	319·49	79·87
3	III	94·74	76·47	93·75	31·58	296·54	74·14
4	IV	93· 5	52·94	94·44	37·50	278·63	69·66
5	Total	345·48	260·66	369·44	194·08	1169·66	..
6	Mean	86·37	65·17	92·36	48·52	Gen. Mean :	73·10

TABLE IV

Analysis of variance of Sin$^{-1}\sqrt{x}$

Due to	D. F.	Sum of squares	Mean square	Remarks
Blocks	3	102·91	34·30	Not significant
Wood *vs.* no wood . .	1	2072·52	2072·52	Highly significant
Lopping *vs.* no lopping .	1	28·35	28·35	Not significant
Interaction . . .	1	211·99	211·99	Not significant
Treatments . . .	3	2312·86	770·95	Highly significant
Error	9	668·66	74·29	
Total	15	3084·43		

It is clear that insertion of buds with wood on has produced a larger number of budlings than insertion of the buds after the removal of wood, the average increase percentage being 32·5. This result is in conformity with that obtained during 1936.

Lopping treatment has not affected the percentage "take".

"*Bud-break*".—The period taken for "bud-break" was collected in every case as in the previous year and results are summarised in Tables V and VI.

TABLE V

Mean number of days (x) *taken for "Bud-break"*

Stock—Kichili *Scion*—Chinee *orange*

Serial No.	Blocks	Treatment No. 1	Treatment No. 2	Treatment No. 3	Treatment No. 4	Total	Mean
1	I	16·00	16·00	9·00	12·00	53·00	13·25
2	II	18·00	17·00	11·00	11·00	57·00	14·25
3	III	13·00	17·00	11·00	12·00	53·00	13·25
4	IV	16·00	16·00	9·00	12·00	53·00	13·25
5	Total	63·00	66·00	40·00	47·00	216·00	..
6	Mean	15·75	16·50	10·00	11·75	Gen. Mean :	13·50

TABLE VI

Analysis of variance of $\sqrt{x}$

Due to	Degrees of freedom	Sum of squares	Mean square	Remarks
Blocks	3	0·0507	0·0169	
Wood *vs.* no wood . .	1	0·1462	0·1462	Not significant.
Lopping *vs.* no lopping .	1	2·0686	2·0686	Highly significant.
Interaction . . .	1	0·0185	0·0185	Not significant.
Treatments . . .	3	2·2333	0·7444	Highly significant.
Error	9	0·3151	0·0350	..
Total	15	2·5991	..	..

Lopping has clearly stimulated an earlier "bud-break". On the average, lopping has effected an earliness of 5·25 days in "bud-break" and presence of wood by 1·25 days.

Maturation of bud-sprouts.—In February 1938, the total extension growths made by the bud-sprouts were collected in the case of all budlings, and these are summarised in Tables VII and VIII.

TABLE VII

Mean extension growth in cm. Nursery Technique Trials

Stock—Kichili *Scion*—Chinee *orange*

Serial No.	Blocks	Treatment No. 1	Treatment No. 2	Treatment No. 3	Treatment No. 4	Total	Mean
1	I	32·60	47·10	40·00	23·67	143·37	35·84
2	II	51·83	25·55	45·13	29·67	152·18	38·05
3	III	61·56	44·50	34·44	48·50	189·00	47·25
4	IV	50·78	55·00	32·54	30·66	168·98	42·25
5	Total	196·77	172·15	152·11	132·50	653·53	..
6	Mean	49·19	43·04	38·03	33·13	Gen. Mean	40·85

TABLE VIII

Analysis of variance for mean extension growth

Due to	Degrees of freedom	Sum of squares	Mean square	Remarks
Blocks	3	303·44	101·15	Not significant.
Wood *vs.* no wood . .	1	121·76	121·76	Not significant.
Lopping *vs.* no lopping . . .	1	444·32	444·32	Not significant.
Interaction	1	1·44	1·44	Not significant.
Treatments . . .	3	567·52	189·17	Not significant.
Error	9	1044·50	116·06	..
Total	15	1915·46	..	..

It is evident that, although earlier lopping produces a slightly less growth of bud-sprouts than the plants lopped at a later stage, the differential effects cannot be termed as significantly greater in favour of any one treatment.

Trial (b)

The summary of the results and analysis of variance of the second trial are presented in Tables IX and X.

TABLE IX

Percentage "take" (x)

Stock—Kichili, *2nd parent* *Scion*—Chinee *orange—2nd parent*

Serial No.	Blocks	Treatment No. 1	Treatment No. 2	Treatment No. 3	Treatment No. 4	Total	Mean
1	I	75·00	58·82	56·25	50·00	240·07	60·02
2	II	87·50	64·28	56·25	18·75	226·78	56·70
3	III	93·75	33·33	88·24	31·25	246·57	61·64
4	IV	81·25	64·23	94·12	[00·00]	239·65	59·91
5	Total	337·50	220·71	294·86	100·00	953·07	..
6	Mean	84·38	55·18	73·72	25·00	Gen. Mean:	59·56

TABLE X

Analysis of variance of $Sin^{-1}\sqrt{x}$

Due to	Degrees of freedom	Sum of squares	Mean square	Remarks
Blocks	3	462·94	154·31	Not significant.
Wood *vs.* no wood . .	1	1858·37	1858·37	Highly significant.
Lopping *vs.* no lopping .	1	377·81	377·81	Not significant.
Interaction . . .	1	40·33	40·33	Not significant.
Treatments . . .	3	2111·91*	703·97	Highly significant.
Error	8	718·36	89·79	..
Total	14	3293·21	..	..

* Separately estimated and not the total of three single degrees of freedom [Yates, 1934].

The above data show that bud-shield with wood has again contributed to a significantly larger percentage " bud-take " than bud-shield without wood, the average increase in favour of the former being 38·96 per cent. Lopping has again had no effect on the percentage " take ".

TABLE XI

Mean number of days (x) *for " bud-break "*

Stock—Kichili, *2nd parent* *Scion*—Chinee *orange—2nd parent*

Serial No.	Blocks	Treatment No. 1	Treatment No. 2	Treatment No. 3	Treatment No. 4	Total	Mean
1	I	13·00	16·00	7·00	8·00	44·00	11·00
2	II	14·00	16·00	10·00	7·00	47·00	11·7
3	III	12·00	13·00	7·00	14·00	46·00	11·50
4	IV	14·00	14·00	9·00	..	37·00	9·25
5	Total	53·00	59·00	33·00	29·00	174·00	..
6	Mean	13·25	14·75	8·25	7·25	Gen. Mean :	10·88

TABLE XII

Analysis of variance of $\sqrt{x}$

Due to	Degrees of freedom	Sum of squares	Mean square	Remarks
Blocks	3	0·0878	0·0293	Not significant.
Wood *vs.* no wood . .	1	0·1526	0·1526	Not significant.
Lopping *vs.* no lopping .	1	2·1959	2·1959	Highly significant.
Interaction . . .	1	0·0001	..	Not si nificant.
Treatments . . .	3	2·4530*	0·8177	Significant.
Error	8	1·0181	0·1273	..
Total	14	3·5589	..	..

* Separately estimated and not the total of the three single degrees of freedom [Yates, 1934].

It is evident that lopping has distinctly stimulated an earlier break of buds, the average difference between lopped and unlopped plants being 6·25.

Presence or absence of wood has not produced any significant difference in respect of "bud-break", in the case of plants lopped in a similar manner.

TABLE XIII

Mean extension growth (in cm.)

Stock—Kichili, *2nd parent* *Scion*—Chinee orange—*2nd parent*

Serial No.	Blocks	Treatment No. 1	Treatment No. 2	Treatment No. 3	Treatment No. 4	Total	Mean
1	I	46·14	52·50	21·70	6·00	126·34	31·59
2	II	37·50	49·25	20·66	7·00	114·41	28·60
3	III	41·92	29·33	23·33	52·50	147·08	36·77
4	IV	34·44	47·00	19·25	..	100·69	27·67
5	Total	160·00	178·08	84·94	65·50	488·52	..
6	Mean	40·00	44·52	21·24	16·38	Gen. Mean :	30·53

TABLE XIV

Analysis of variance

Due to	Degrees of freedom	Sum of squares	Mean square	Remarks
Blocks	3	207·76	69·25	Not significant.
Wood *vs.* no wood . .	1	0·12	0·12	Not significant.
Lopping *vs.* no lopping .	1	2199·52	2199·52	Significant.
Interaction . . .	1	88·04	88·04	Not significant.
Treatments . . .	3	2287·68	762·56	Not significant.
Error	9	1875·21	208·36	..
Total	15	4370·65	..	..

A distinctly larger extension growth is observed in plants which are lopped after the bud-sprouts have produced some growth, the average increase in extension growth in favour of unlopped plants being 23·45 cms., and the critical difference 17·07. Presence or absence of wood has not significantly affected the extension growth.

In general, it is possible to state on the basis of these two trials that :—

(*i*) the presence of wood in the orange bud-shield has contributed to over 32 per cent higher " take " of buds on *kichili* rootstocks ;

(*ii*) lopping of *kichili* rootstocks at the time of bud-insertion has not affected the percentage " take " of orange buds ;

(*iii*) lopping of *kichili* rootstocks has stimulated an earlier "bud-break" by over five days in *chinee* orange ; and

(*iv*) presence or absence of wood in the *chinee* orange bud-shield has shown to exert no material influence on the extension growth of bud-sprouts on *kichili*.

Trial (c)

The data on " bud-take " of acid lime scion parent are presented in Tables XV and XVI.

TABLE XV

Percentage " take " (x)

Stock—Kichili, *2nd parent* *Scion—Acid lime*

Serial No.	Blocks	Treatment No. 1	Treatment No. 2	Treatment No. 3	Treatment No. 4	Total	Mean
1	I	64·29	23·08	30·00	0·00	117·37	29·34
2	II	75·00	14·28	50·00	33·33	172·62	43·15
3	III	76·47	12·50	41·18	16·67	146·82	36·71
4	IV	73·33	33·33	25·00	10·00	141·66	35·42
5	Total	289·09	83·19	146·18	60·00	578·46	..
6	Mean	72·27	20·80	36·55	15·00	Gen. Mean :	36·15

TABLE XVI

Analysis of variance of $\sin^{-1}\sqrt{x}$

Due to	Degrees of freedom	Sum of squares	Mean square	Remarks
Blocks	3	58·98	19·66	Not significant.
Wood *vs.* no wood . .	1	1682·22	1682·22	Highly significant.
Lopping *vs.* no lopping .	1	452·22	452·22	Significant.
Interaction . . .	1	389·11	389·11	Significant.
Treatments . . .	3	2596·40*	865·46	Highly significant.
Error	8	394·47	49·31	..
Total	14	3049·85	..	..

* Not the total of the three single D. F. but separately estimated [Yates, 1934].

In this scion, the unlopped stocks worked with wood attached to the bud-shield has produced an outstandingly larger " take " of buds than in any other treatment, the increase over any other treatment being 35·72 per cent or over.

Presence of wood has contributed to 36·51 per cent higher " take " of buds than absence of wood, while unlopped plants have produced 20·76 per cent higher " take " than the lopped ones.

TABLE XVII

Mean number of days (x) *for " bud-break "*

Stock—Kichili, *2nd parent* *Scion—Acid lime*

Serial No.	Blocks	Treatment No. 1	Treatment No. 2	Treatment No. 3	Treatment No. 4	Total	Mean
1	I	17·00	31·00	11·00	..	59·00	14·75
2	II	20·00	24·00	15·00	15·00	74·00	18·50
3	III	20·00	29·00	15·00	14·00	78·00	19·50
4	IV	18·00	28·00	16·00	..	62·00	15·50
5	Total	75·00	112·00	57·00	29·00	273·00	..
6	Mean	18·75	28·00	14·25	7·25	Gen. Mean :	17·06

TABLE XVIII

Analysis of variance of $\sqrt{x}$

Due to	Degrees of freedom	Sum of squares	Mean square	Remarks
B'ocks	3	0·0965	0·0322	Not significant
Wood *vs.* no wood . .	1	0·8032	0·8032	Not significant
Lopping *vs.* no lopping .	1	3·3594	3·3594	Highly significant
Interaction . . .	1	0·6781	0·6781	Significant
Treatments . . .	3	5·4669*	1·8189	Highly significant
Error	7	0·5220	0·0746	..
Total	13	6·0754	..	..

* Separately estimated and not the total of the three single D. F. [Yates, 1934].

As in the case of orange scion, lopping of rootstocks has stimulated an earlier " bud-break " by an average of 12·63 days than in the unlopped series. Presence or absence of wood does not appear to be a factor which influences the " bud-break " period. Since the number of successfully budded plants under treatment No. 4 is very small and nil in two plots, these large differences may not present a very accurate picture of the effects of lopping on " bud-break ".

As a large number of acid lime bud-sprouts failed to make a good growth and, further, as no bud-sprouts were available on two plots, it was not possible to analyse the results relating to the extension growth made by the plants under this experiment. The following inferences which are drawn from this experiment are, therefore, confined to only the data collected on " bud-take " and " bud-break ".

(*i*) Presence of wood in acid lime bud-shield has contributed to over 36 per cent higher " take " of buds on *kichili* rootstocks.

(*ii*) Lopping of *kichili* rootstocks at the time of bud-insertion has lowered the " take " of acid lime buds by about 20 per cent.

(*iii*) Presence or absence of wood in acid lime bud-shield has not had any effect on the period taken by buds to " break " on *kichili* rootstocks.

(*iv*) Lopping of *kichili* rootstocks has stimulated an earlier " bud-break " in acid limes by an average of over twelve days.

Discussion

Taking the results of all the three trials carried out during 1937-38 into consideration, it is observed in general that the results confirm those obtained in the preceding year, in so far as the beneficial effects of the presence of wood in the bud-shield is concerned. The practice of lopping at the time of bud-insertion has also shown both in the 1936-37 as well as in 1937-38 trials to have a distinctly stimulating effect on the "bud-break". With regard to the extension growth of bud-sprouts as effected by lopping treatment, the results of the two years and also those between the different series in the 1937-38 trials are contradictory to a certain extent. However, when it is remembered that the delayed lopping of rootstocks in all the four months in the 1936-37 trials and in the second trial with *chinee* oranges in 1937-38 have produced significant increases in the extension growths of bud-sprouts over the method of lopping the rootstocks at the time of bud-insertion, and also the extension growth of the bud-sprouts in the first trial in 1937-38 has been higher though not statistically significant in the unlopped series than in the lopped, it appears reasonable to assume that delayed lopping is conducive to an early maturation of bud-sprouts.

Mendel [1937] has shown that the lopping of the rootstocks results in much more serious injury to the plant than the wound caused by the 'T' cut, because of the interference with the supply of the photosynthetic products from the side of the stub. Delayed lopping would therefore appear to be a practice more in consonance with the plant requirements at this stage than lopping at the time of, or immediately after, bud-insertion.

Considering that the removal of wood from bud-shield or the removal of the shield without the wood attached to it requires time and skill and that the removal of the wood involves some risk of injury to the bud, it is suggested that the replacement of the prevalent Indian method of removal of wood from the bud-shield by the American method of retaining the wood will be of considerable practical and economic importance in citrus nursery practices.

Both during 1936-37 and 1937-38, the trials carried out definitely establish the fact that July, August and September provide the optimum period for budding oranges in this tract. The data also seem to reveal that, for securing the most favourable results, budding should be carried out during this period when dry weather prevails. October to January provides the second best period for budding operation, provided the north-east monsoon does not cause heavy precipitations during the budding period and the hot and dry weather does not follow immediately after bud-insertion, as it usually happens in the case of January bud-insertions. If the hot season is not prolonged up to the end of June, a fairly high "take" of buds can be reasonably expected in that month also.

The above results are not in accord with the experiences of the citrus nurserymen in most parts of India. In the matter of the influence of presence of wood in the bud-shield, the results obtained in England [Davies, 1917]

are also contradictory to those obtained in these trials. Garner [1934] has, however, recognised the possibility of seasonal conditions affecting the results and has stated that it has been found necessary in England to insert apple and plum buds with wood attached, because of the conditions of the scion variety, which prevented the removal of wood without injury to the bud. He also points out that the difference in " bud-take " obtained in his trials is very small and unlikely to be of economic importance. In fact, he considers that insertion of bud-shield with wood will lead to economy of time, reduce loss and furnish a more useful method to the amateur budder of apples, pears, plums and cherries.

It is possible that the results may be affected to a considerable extent by the age of the rootstocks, nature of scion wood, skill of operators, period of exposure of the bud-shield prior to insertion and also the nature of the material or solution in which the bud-wood and the separated bud-shield is retained till the time of actual insertion. It may be pointed out that the slice of wood when retained in the bud-shield probably serves a useful purpose in retaining moisture in the bud for a longer period than when without it. Opinion is also likely to be expressed that the wood if retained leads to the interposing of a foreign body between the healing the surfaces, thus possibly preventing the bark of the bud from uniting directly with the exposed surface of the rootstock. Bailey [1920] holds that the youngest portions of the wood in the bud probably unite with the stock, but such union may not be possible when knife cuts are deep and denser part of wood or pith of the trees are retained in the bud-shield. Microscopic examination of the eighty-three transverse sections of the bud-joint of the plants budded with wood on in these trials after about five months of the insertion has not shown the presence of any interposing body between the cambium layers. It, therefore, appears clear that when citrus rootstocks of about twenty months age are budded with bud-shields having a slice of wood, no impediment occurs in the complete union of the two cambium layers.

It has, however, been found that, where buds with very thick slices of wood are inserted, the bud-joint becomes abnormally raised out, forming a gall-like structure. When such galls are very large, the plants die a premature death(Plate XXXI, fig. 2). The size of the wood retained in the bud-shield in the present trial was very small, with a smooth and flat cut surface, not exceeding 2·50 cm.×0·40 cm.×0·15 cm. The size was the same as was obtained by the operator at the time of slicing out from the bud-stick. The operator was prevented from inserting any bud-shield which was not found to the mark or in which case the size of the attached wood had to be reduced after slicing out from the bud-shield. These show that the operator has to be trained sufficiently in order to be able to skilfully slice out the bud-shield of the required kind. Lack of skill on the part of operator, unsuitability of the season and unfavourable treatments given to the bud-shield prior to insertion are, therefore, likely to be a few of the more potent causes for the divergent experience of the citrus nurserymen with regard to the relative merit of bud-insertions with and without wood attached to the bud-shield.

FIG. 2

FIG. 1

The formation of a callus pad at the lower end of the inverted 'T' cut caused by exuberant growth has been referred to by Mendel [1937] in his investigations on anatomy and physiology of citrus budding. In properly budded plants, the callus beneath the bud-shield has been shown to cease its growth as soon as the gap between the rootstock and the shield is filled and the resulting pressure renders further cell division impossible. The callus may, however, grow into the cleft formed at the horizontal cut on the rootstock without hindrance from the tissues lying above, thus forming a pad. It is possible that when a rough and ununiform slice of wood is retained in the bud-shield or when the horizontal cut is too large or the bud-shield is ill-fitting, the callus pad may assume large size. If a connecting cambium differentiates in such a pad, as it is reported to do two to three weeks after budding [Mendel, 1937], the activity of this cambium may lead to the formation of more cell layers resulting in the raising out of the bud-shield and formation of a swollen bud-joint instead of a smooth one as shown in Plate XXXI, fig. 1.

That the common practice of the budders to retain the bud-shield in water or inside the mouth of the operator in contact with saliva hinders successful " take " of such buds has been a widely accepted belief. In the experiments reported here, all the bud-shields were separated out immediately before insertion and inserted with the minimum of exposure and without contact to any kind of liquid or storage in any other material or container.

Investigations on the anatomy and histology of citrus budding in Palestine [Mendel, 1937] has shown that, while the outer layers of wood are able to form callus easily, the wounds which penetrate deeper into the wood are closed by only one overgrowth of the callus tissue originating from the peripheral regions of the wounds. This shows that in the hands of an inexperienced budder, the extent of activity of the healing tissue and consequently the success of the bud-union will depend upon the regenerative power of the peripheral regions of the inflicted wounds. It has also been shown in the above-mentioned investigation that the layers between the primary bark and the pith in young citrus rootstocks are not sharply defined, so that when the bark is lifted for budding, it is never separated from the stem exactly at the inner border of the cambium. It would also appear that in the process of making the 'T' or inverted 'T' cut on the rootstock, the cut may reach the interior sapwood still in the initial lignified state, which has not the power to regenerate. Insertion of the bud with a thick slice of wood adhering to it or a careless incision are, therefore, points that may make all the difference between the success and failure of the insertion.

The data set forth in the preceding pages regarding the optimum season for orange budding on *kichili* and *gajanimma* has shown that the latter variety of rootstock produces a higher " take " of buds than the former. The wide difference existing between the various citrus varieties regarding the efficiency of "bud-take " has also been proved in a number of other trials which have not been reported in these pages. These facts go to show that the practical interests of a citrus nurseryman and the grower are not necessarily

identical. While the former may raise oranges on a rootstock which produces the maximum " bud-take ", the latter may find that the variety favoured by the nurserymen may not give the best performance. In fact it has been found at this station that, of all the citrus varieties used as rootstocks, *gajanimma* is the most susceptible to gummosis, while *kichili* appears to be among those that are fairly immune to this disease. In order to select the best rootstock from the viewpoint of the grower and nurseryman elaborate rootstock trials are, therefore, necessary to supplement the results from the nursery technique trials.

Summary

1. Presence of wood in *chinee* orange and acid lime bud-shield has shown to produce a significantly higher " take " of buds on different rootstock varieties than absence of wood.

2. Primary lopping of citrus rootstocks at the time of bud-insertion has lowered the " take " of buds in some cases.

3. Primary lopping of the citrus rootstocks at the time of bud-insertion has stimulated an earlier " bud-break " both in *chinee* oranges and acid limes.

4. Delayed primary lopping of citrus rootstocks, after the inserted *chinee* orange buds have produced not less than two inches of extension growth, has resulted in a comparatively rapid extension growth of bud-sprouts.

5. Presence or absence of wood in the *chinee* orange and acid lime bud-shields has not affected the period taken for " bud-break " or for rapid extension growth of *chinee* orange bud-sprouts.

6. July to September provide the optimum season for budding *chinee* oranges in this tract, while October to January provide the next best period, provided the bud-insertions are done sufficiently before the advent of the hot-weather period. Heavy precipitation during bud-insertions appear to inhibit the " take " of buds.

7. *Gajanimma* produces a significantly higher " take " of buds than *kichili*. This, however, cannot be taken to mean that the former is a more suitable rootstock than the latter.

Conclusion

For producing a larger number of budded plants of *chinee* oranges and acid limes and for encouraging an early maturation of bud-sprouts, insertion of bud-shield with a piece of wood attached to it and primary lopping of the rootstocks after the bud-sprouts have produced not less than two inches of extension growth, have been found to be the most suitable practices.

July to September has proved to be the optimum period for budding *chinee* oranges under the conditions prevailing in this tract. Poor " take " has been shown to result when bud-insertions are made under wet-weather conditions or during hot and dry spells. Hot season, if it follows bud-insertion very soon, has been shown to cause injury to bud-sprouts and retards their subsequent growth.

A higher " take " of orange buds is obtained on *gajanimma* than on *kichili*.

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EXPERIMENTS ON THE EFFECT OF X-RAY ON *PENNISETUM TYPHOIDEUM* RICH., *NICOTIANA TABACUM* LINN. AND *BRASSICA JUNCEA* HK. f & T

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I. Introduction

Since Muller's [1927] important work, several investigators have studied the effect of X-rays on plants and animals. Species of *Nicotiana* and *Drosophila* are among the materials frequently used for irradiation experiments. Treating five species of *Nicotiana* with X-rays and radium, Goodspeed and Olson [1928] observed that the effect produced in all the species was that the progenies showed variation, some of the variations resulted from disturbances during mitotic and meiotic divisions and many of the variant characters in the progeny of X-rayed plants were inherited. Later Goodspeed [1929] had found that the effect of X-rays on *Nicotiana sylvestris* was similar to that on *N. tabacum* in producing variable quantitative chromosomal alterations which gave rise to series of by-products in later generations. Stadtler [1930] working on barley and maize found that the rate of induced mutation in germinating seeds was four times as much more as those in dormant seeds. When the dose applied to the dormant seeds was doubled the rate of mutation approximately doubled. The double dose applied to germinating seeds was almost completely lethal in effect. Increase in moisture

content and variations in temperature had no effect on rate of induced mutation. The gene mutations obtained by Stadtler were all of recessive type. Chromosomal reorganization observed by Goodspeed in tobacco was also observed by Stadtler in the case of barley and maize. Goodspeed and Avery [1934] obtained from the progeny of a single X-rayed female gamete of *N. tabacum* fourteen types. Seven of these were pure breeding types and the remaining seven not established as pure. These types differed from one another and the control in habit, form of leaf, flower and capsule, and in colour of leaf and flower. Chimeral effects as a result of exposure to high frequency waves were observed in barley and maize by Stadtler and in tobacco by Goodspeed. By subjecting the seeds of broad bean, barley and mustard to same condition and dosage which resulted in different effects, Patten and Wigoder [1929] concluded that a specific dose is required for each. Stunting followed in all cases where plants growing for twenty-four hours were rayed. Difference in sensitiveness between shoot and root was noted in barley. With the dosage used the only detrimental effect observable in mustard was the failure of side roots to develop. Shull and Mitchell [1933] working on wheat corn, oats, etc., found that brief exposure to X-rays produced from high voltage and low ampereage had a stimulative effect on plant growth. Using stronger dose of 300 r-units Moore and Haskins [1934] observed physiological variation and abnormalities including dwarfing, delayed growth and fasciation in leaves of cotton, tung-oil and other plants. Ramiah and Parthasarathy [1936] obtained an ageotropic mutant in rice by exposure to weaker dose. Singh and Choudhri [1935] noted that exposure of dry seeds of *N. tabacum* to X-rays for one minute had the effect of inducing physiological, morphological and chemical variations.

The authors have made no attempts to review here all the previous work done on X-raying plants but have referred to literature that was accessible to them.

II. Material and method

The first attempt to study the effect of irradiating crop plants with X-rays in Bombay Province was made by Dr. W. Burns in August 1931 and the work was later continued by the present authors. Through the courtesy of Major Chambers, Specialist in Radiology, the plants were rayed at the Connaught Military Hospital, Poona, for the first two years and later with the permission of the authorities the raying experiments were continued at the David Sassoon Hospital, Poona, for the remaining period. Because of the limited facilities available and because of the clinical purposes for which the X-ray apparatuses were used in the hospitals the irradiation experiments had to be restricted in scope and they could not be carried out to their conclusion.

Bajri (*Pennisetum typhoideum*), the bulrush-millet, mustard and tobacco were the materials used for the experiments. The result of each of these plants is discussed separately.

(*a*) *Bajri* (*Pennisetum typhoideum*)

The difference in time of maturity of the male and female flowers of *bajri* inflorescence was found suitable for irradiating the male and female gametes separately. The receptive stigmas are first extruded, followed a couple of days later by the first wave of mature anthers. The second wave of anthers ripens and becomes extruded from the glumes still a few days later. A few plants of a pure strain 54 of *bajri* were grown in earthen pots and when the earheads were ready for treatment, were removed to the hospital for raying. The male and female gametes were subjected to X-rays by exposing the ovaries and anthers respectively. The ovaries and anthers were separately rayed and pollination effected after the plants were brought back to the garden. The plant materials were rayed with a metalax water-cooled tungsten target X-ray tube. The dose and the period of exposure to which the earheads were subjected is given in Table I.

TABLE I

Dosage used for raying the ovaries and anthers of P. typhoideum

Experiment No. 1, 1931

——	K. V.	M. a.	Time of exposure in minutes	Target distance in inches
Dose 1	60	5	5	14
Dose 2	50	5	5	30 c. m. s.
Dose 3	62	5	5	14
Dose 4	45	5	5	14

The above doses were all applied without the use of a screen. The only two variables were distance and K. V., the other conditions were common to all. The pollination effected between rayed and unrayed material and the number of grains set in each earhead is given in Table II. The earheads were protected by paper bags and the grains produced were sown in the following season. For two generations selfed progenies of the irradiated plants were kept under careful observation.

TABLE II

Condition of the material used for pollination and the number of grains set

Earhead No.	Stage of development and treatment given		No. of seeds set	Remarks
	Ovary	Anther		
1 . .	mature unrayed .	mature rayed .	491	
2 . .	Do. . .	Do. . .	0	
3 . .	Do. . .	Do. . .	159	The average number of grain produced in a single earhead of *P. typhoideum* naturally grown is 1204.
4 . .	Do. . .	Do. . .	150	
5 . .	Do. . .	Do. . .	698	
6 . .	Do. . .	Do. . .	16	
7 . .	Do . .	immature rayed .	44	
8 . .	Do. . .	Do. . .	1099	
9 . .	mature rayed .	mature unrayed .	95	The average for rayed earheads is 381 grains.
10 . .	immature rayed .	Do. . .	1202	
11 . .	mature rayed .	Do. . .	248	
		Total .	4202	

From Table II it is observed that in one instance no seeds were set at all and in many setting is very poor. Only in two instances out of eleven the setting is normal. The seeds from the eleven earheads were sown in ear to row cultures in the following season.

The result of sowing showed that only in one instance (earhead No. 6 of Table II)—grains produced by pollination of unrayed earhead with rayed pollen—there was total failure of germination. In all other cases there was no indication of any adverse effect produced as a result of raying. The germination percentage of grains in all cases including the only two controls provided was exceedingly low. Provision of insufficient controls had made it difficult to draw definite conclusions. Except for the poor setting of grains in the majority of plants either of whose gametes were rayed (Table II) and the low percentage of germination observed while trying to raise the F_1 generation, no other abnormal or off-types could be noticed in any of the cultures of plants raised from treated materials.

Table II shows that in the majority of instances of earheads with either the male or female part mature and subjected to X-rays, the grain setting has been deficient as compared with the average number of grains produced in earheads grown under natural conditions.

In 1932 in addition to the exposure of ovaries and anthers to X-rays, germinating seeds of *P. typhoideum* were also rayed. The seeds treated were previously soaked in water for twenty-four, forty-eight and seventy-two hours. The following doses were applied to seeds of each of the three different periods of soaking as well as to ovaries and anthers.

TABLE III

Frequency of X-rays to which soaked seeds and earheads of P. typhoideum *were exposed*

Treatment No.	Distance in inches	Filter	K. V.	M. a.	Exposure in minutes
1 . . .	12	*Nil.*	50	5	10
2 . . .	12	*Nil.*	50	5	15

Since the X-ray apparatus at the hospital was small and not powerful enough, only few grains could be rayed at a time. Therefore, the data presented is that based on a small population and is not capable of being subjected to too critical statistical interpretation. The result of plants raised from rayed germinating grain is given in Table IV.

TABLE IV

Difference in growth effect between the progeny raised from soaked X-rayed and control grains of P. typhoideum.

Character studied	Rayed	Control	Difference
Yield of grain in grams	7·7	10·3	=2·6
Height of plant in feet	4·7	4·9	=0·2
Number of tillers per plant . . .	3·6	4·5	=0·9
Length of earheads in inches . . .	5·5	4·8	0·7

From Table IV it will be observed that plants raised from rayed grains show slight weakening as compared with the control except for the character of length of the earheads. No difference in result was observable between

grain soaked for different number of hours and later subjected to X-rays for varying periods. Since application of stronger doses was impracticable, further work on raying *P. typhoideum* had to be discontinued.

As in the case of earheads of *P. typhoideum* treated in 1931 so also in those rayed in 1932 the setting of grain was poor. The untreated controls produced normal well-set earheads. The grains that were set in rayed earheads when sown gave rise to normal progeny which resembled the controls for all characters. The result, therefore, of X-raying earheads of *P. typhoideum* to variations in unfiltered rays produced from 45 to 62 K. V., 5 M. a., at twelve to fourteen inches target distance from five to fifteen minutes had neither deleterious nor stimulative effect.

(*b*) *Mustard* (Brassica juncea *Hk.f. & T.*)

The apparatus used for X-raying mustard seeds was Victor Wanz Junior fitted with a coolidge air-cooled tungsten target X-ray tube. The dosage applied was 80 K. V., 5 M. a., target distance three inches, without filter. Dry seeds of mustard were exposed for nine minutes by three intermittent application of three minutes each since it was not possible to give continuous application.

The rayed seeds were sown in plots with suitable controls. The rayed and control materials were replicated seven times by A. B. B. A. method. The result of observations of the plants from rayed seeds is given in Table V.

TABLE V

Difference between rayed and unrayed dry seeds of mustard

Observation	Rayed material	Control	Difference	Significance of difference
Average No. of fruiting branches	7·67	8·40	=0·73	Not significant
Height in feet . . .	5·23	5·29	=0·06	Do.
Seed yield in grams. . .	4·13	5·73	=1·60	Do.

Although the above figures show that difference between the rayed and unrayed is not significant for any of the character studied yet a slight weakening of the plants raised from dry seeds exposed to X-rays for nine minutes is clearly noticeable.

(*c*) *Tobacco* (Nicotiana tabacum *Linn.*)

Both dry and soaked seeds of tobacco were exposed to same frequency of rays as that for mustard and the duration of exposure was limited to three minutes. The rayed seeds were sown in plots along with controls replicated

five times by A. B. B. A. method. The results of observations made on certain characters are given in Tables VI and VII for soaked and dry seeds respectively.

TABLE VI

Difference between rayed and unrayed soaked seeds of tobacco

Characters studied	Rayed material	Control	Difference	Significance of difference
Average No. of leaves per plant .	10·12	11·14	=1·02	Significant
Height of plant in inches . .	2·63	3·06	=0·43	Do.
Average leaf factor of biggest leaf	76·71	96·28	=19·57	Do.

The above comparison between the plants from soaked seeds exposed to X-rays and controls shows that the difference in respect of all the three characters studied is significant. Soaked seeds have, therefore, showed a general but significant weakening.

TABLE VII

Difference between rayed and unrayed dry seeds of tobacco

Characters observed	Rayed material	Control	Difference	Significance of difference
Average No. of leaves per plant .	9·91	10·51	=0·60	Not significant
Average height of plant in feet .	2·74	2·86	=0·12	Do.
Average leaf factor of biggest leaf	82·17	92·66	=10·49	Do.

From the figures given in Table VII it is observed that in the case of dry seed of tobacco the same treatment as that given to soaked seeds had slight but not significantly weakening effect. A comparison of the results of soaked and dry seeds of tobacco exposed to the same duration and dosage of X-rays shows that the deleterious effect observable in the weakening of plants is more pronounced in the case of soaked than dry seeds.

In 1934 the previous years' experiments were repeated but owing to a severe attack of damping-off disease both the control and rayed material were totally lost. These experiments could not be continued any further since the only apparatuses that could be employed were those in hospitals used for clinical work and were not powerful enough to induce changes abnormal in character.

A comparison of the effect of X-raying the aforementioned three crops seems to indicate that the dosages used have not produced any deleterious effect in any of them except one. Even the stronger dose used in the case of dry seed of mustard (80 K. V., 5 M. a., 3 in. distance and 9 minutes exposure) as compared with others has not produced any weakening while the same dosage used for only three minutes produced significant weakening effect on soaked seeds of tobacco. It was observed that the power of germination of rayed seeds, whether dry or soaked at the time of treatment, was generally affected. Treated seeds showed poorer germination as compared with the control. In Table VIII is given in a summarised form the results of raying the three crops used in these experiments.

TABLE VIII

Summary of results of raying given in tabular form

Crop	Condition of part rayed	K. V.	M. a.	T. distance in inches	Exposure in minutes	Result
Mustard .	Dry seed .	80	5	3	9	No appreciable weakening
Tobacco .	Dry seed .	80	5	3	3	Weakening not significant
Tobacco .	Soaked seed	80	5	3	3	Significant weakening
Bajri .	Soaked seed	50	5	12—14	5—15	No appreciable weakening
Bajri .	Earhead .	50	5	12—14	5—15	No appreciable weakening

III. DISCUSSION

Stadtler [1930] observed in the case of barley and maize and Goodspeed [1929] in the case of tobacco that soaked or germinating seeds were more adversely affected by X-rays than dry seeds. This is confirmed by the results obtained on raying mustard and tobacco. Nine minutes exposure had no deleterious effect on dry seeds of mustard while the same dose applied for three minutes in the case of tobacco had no effect on dry seeds but significantly weakening effect on soaked ones. Patten and Wigoder [1929] found that specific doses were necessary for different crops to induce changes. The result of the three crops used in these experiments lends support to this conclusion.

Singh and Choudhri [1935], exposing *Nicotiana tabacum* seeds to minute dose of X-rays for one minute, were able to induce physiological, morphological and chemical variations conducive to accelerated metabolic activity of the

plant, while the authors using the same material (*N. tabacum*) but slightly stronger dose obtained a very different result, *viz.*, a general weakening of the plant. Shull and Mitchell [1933], in whose experiments stimulative effect resulted in seedlings treated with mild dose of X-rays, state that deleterious effects are consistently obtained when unfiltered radiations are used. This explains the difference between the results of Singh and Choudhri [1935] and the present authors. In the latter's case no filter was used and consequently whatever beneficial or stimulating effect there might have been, was masked by the deleterious effect produced from unfiltered rays.

Moore and Haskins [1934], Ramiah and Parthasarathy [1936], and Singh and Choudhri [1935] irradiated dry seeds ; Shull and Mitchell [1933] Noguchi [1935] and Benedict and Kersten [1934] treated soaked seeds. These workers, however, have not compared the results of X-raying dry and soaked seeds of the same plant. The authors tried the effect of unfiltered X-rays on both dry as well as germinating seeds of *N. tabacum*. The difference in effect between these two treatments was that in the case of plants raised from irradiated dry seeds the general weakening was not significant as compared with the controls while for those plants raised from irradiated soaked seeds the difference due to weakening was significant. From the results it may be suggested that unfiltered rays produce more deleterious effect in the case of soaked than dry seeds. These results somewhat compare with the results of Goodspeed who observed that seeds of *Nicotiana* were resistant and seedlings susceptible to effect of high frequency radiation. Stadtler [1930] found that dry seed could stand ten times the dose to which germinating seeds were subjected to.

In order to induce mutations it appears that strong dosage is necessary. None of the doses applied proved lethal to the millet, mustard or tobacco used in the experiments.

IV. Summary

(1) The effect of X-raying ovaries and anthers of *P. typhoideum* with a weak dose of unfiltered X-rays produced neither deleterious nor stimulative effect.

(2) Germinating seeds of *P. typhoideum* exposed to the same dosage of X-rays as the ovaries and anthers resulted in the weakening of the progenies raised. But the weakening was not statistically significant.

(3) Mustard and tobacco seeds when rayed showed a general loss in vigour of growth. The weakening was significant in the case of plants raised from irradiated soaked seeds and not significant in the case of those raised from irradiated dry seeds.

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REVIEWS

Statistical Technique in Agricultural Research. By D. D. Paterson (First Edition—McGraw-Hill Publishing Company, Aldwych House, London, W. C. 2). Pp. 263. Price 18*s*.

This addition to the already considerable number of commentaries on statistical method is welcome, as the examples selected are simple and illustrative of statistical principles.

Chapter I deals with general principles, the need for the reduction of data, calculation of mean, sum of squares and standard deviation [why the sum of squares should be divided by (n-1)], the principle of normal curve of errors, the principle of statistical significance and Fisher's t test. Short methods of computation in each case have been illustrated in a popular way. The author might have mentioned at this stage the idea of " Fiducial limits " introduced by Fisher.

Chapter II deals with the analysis of variance, and explains in a routine way how to calculate the sum of squares and the variance due to each item. Fisher's z test has been well explained, but the author might have stressed that when some treatment differences are considerably larger than others, it is necessary to separate " the errors " into different sets, instead of using a combined analysis and have a single " error " as a basis for comparison.

Chapter III deals with ' Goodness of Fit ', though as a logical sequence, it should have preceded the analysis of variance. The fact that χ^2 and t distributions are special cases of that of z—a fundamental idea in dealing with these distributions, has not been stressed in this publication anywhere clearly. The examples given for illustrating the χ^2 test are well known, being commonly employed in preparing contingency tables connected with genetical problems ; but it would have been better to refer in detail to the additional aspect of χ^2 and to the correction necessarily made when frequencies are discontinuous or are very small which often happens at the tail end of the distribution.

Chapter IV dealing with " diagrams " is scanty as it has not touched upon any cases of asymmetrical curves.

Chapters V and VI deal with correlation and regression respectively, their separation is hardly necessary. The idea of regression in view of its increasing importance should have been given greater prominence.

Chapters VII to IX dealing with new designs of Field Experiments are of great interest to the agricultural experimenter. The author has rightly stressed that the first essential for good experimentation, which is often forgotten, is sound crop husbandry, *i.e.*, a detailed knowledge of the crop, soil, manurial requirements, cultural technique, etc. Then the site selected for the experiment should be as uniform as possible. The nature of experimental problems should be clearly defined, and the treatments should be so chosen as to have a common basis for comparison and as to give fairly wide differences in yields between them. Every agronomist now knows that for a valid interpretation of his experimental results, the size of plots, their arrangement and shape, number of replications, all contribute their share, and that even in adopting the usual randomised-block method or Latin square, the greatest care in design is necessary to obtain a valid estimate for error.

Chapter VIII deals with serial and perennial crop experiments. The author has treated the subject in a popular way. It should, however, have been emphasised that for such a combined analysis of variance it is necessary, that error variances in the several seasons should not differ significantly if there should be any such difference the precision in the several seasons should be equalised by suitable weights. With regard to designs for perennial crops (such as tea or fruits), the author has rightly stressed that each plot should contain at least ten trees and that suitable guard row should be provided for (double row or single row depending upon the field available).

Chapter IX deals with some of the latest designs recently issued from Rothamsted and on which F. Yates has already published his bulletin (Imperial Bureau of Soil Science—Technical Communication No. 35—The design and analysis of Factorial Experiments). The analysis of a complex experiment, and that of the split plot design and the analysis of covariance have been explained in detail, but only a brief reference has been made to the " confounded layouts " possible in 2^3 and 3^3 designs.

This book will help towards a clear understanding of modern statistical ideas and will be found useful by the agricultural experimenter for whom it is meant.

Introduction to the Botany of Field Crops. 2 Volumes. (South African Agricultural Series, Vol. XVI). By J. M. Hector, Professor of Agricultural Botany, University of Pretoria. Pp. 1127, with 448 Text-figures and 88 Tables. Published by the Central News Agency Limited, Johannesburg, South Africa. Price £3 10*s*. 0*d*. net per set of 2 Volumes (postage 2*s*. extra).

The author in these two volumes has presented in a co-ordinated form the fundamental facts and principles relating to the structure, functions and life-relations of plants. A considerable advance has been made in the knowledge of botany of cultivated plants during the last two decades and investigations on a number of new lines have been undertaken. The literature during this period has also become extensive and widely scattered. The necessity for collecting and co-ordinating this information is manifest and the author has attempted to meet that demand. The study of structure being fundamental to a proper understanding of all other aspects of plant study, morphology is emphasised throughout. But since structure has significance mainly in terms of function, the principal physiological processes are also given a considerable attention. To obtain a fuller understanding of the field crops, approach has also been made from other angles, *i.e.*, cytogenetics, ecology and systematics. It is only by so doing that it is possible to understand a living creative organism. It not only gives a more thorough insight into the behaviour and reactions of plants in the field but leads to a better understanding of the potentialities of crop production.

The book is divided into two volumes —Volume I dealing with cereals and Volume II with other crops. The division, however, is not quite so absolute since Volume I also deals with non-cereal crops such as sugarcane. The examples of plants selected by the author for demonstrating various phenomena are taken from field and garden crops. In the description of plants generally the structure and functions of the vegetative organs of plants precede that of the organs concerned with reproduction. The seed development in most cases is well explained and fully illustrated but the conditions favourable to germination of seed are not stated. A description of the conditions required for successful germination of seeds of crops like sugarcane, tobacco, lucerne, berseem, etc. would have been useful. In cereals a statement of comparative nutritional value of millets and other grains would have greatly enhanced the value of the description of these crops. The chapter on maize, however, is comparatively more complete giving the economic uses of maize and food constituents of the grain.

One would normally not expect results of agronomic and fertiliser trials on the growth of plants in a botanical treatise of this nature but, since the author has given such results in the case of cotton, it would have been just

as well to give similar results for other crops to make the book more complete. In relation to Indian work, references to recent investigations on crop husbandry in different schemes of the Imperial Council of Agricultural Research could have been usefully added to bring the knowledge up-to-date.

The author has rightly paid greater attention to those Natural Orders which include important agricultural crops, for instance, the Leguminosae and the Malvaceae, and has scantily dealt with those such as the Compositae which, though large, are not so important agriculturally. The Leguminosae occupies the largest space covering 167 pages with the Malvaceae coming next with 114 pages. The description of these two Orders is of the nature of a running commentary of botanical investigations on the different families. The chemistry of nitrogen fixation by the different plants of the Leguminosae and the differentiation on morphological and physiological grounds of organisms causing nitrogen fixation are useful contributions to knowledge. The chapters on the Solanace, the Cucurbitaceae and Liliaceae give a description of plants, the structural knowledge of which will be found useful by those interested in vegetable gardening. In dealing with potato, the author has described the quality in various types. Useful findings in regard to the effect of light on germination of potato are recorded but perhaps an account of relative usefulness of practical methods of preservation of seed potatoes would have been a more useful contribution. In taxonomic studies the author has rightly laid stress on the necessity of a change in the outlook of methods of classification and advocates the principles of adoption of new units of differentiation based on genetic variability. This principle has already been expounded by the Imperial Council of Agricultural Research in the paper entitled " The Description of Crop-plant Characters and their Ranges of Variability—(*i*) in Cotton and (*ii*) in Rice " [*Ind. J. Agric. Sci.* Vol. VIII, Part V, p. 567.]

The book is comprehensive in scope including material from all the divisions of botany. It is written neither merely as a text book in the accepted meaning of the term nor is its subject matter intended for any particular course. It forms a useful compendium of botanical investigations on cultivated crops and will thus be found useful by botanists and agronomists undergoing post-graduate training. The author has given references to relevant literature containing detailed accounts of various researches mentioned in the text. At the end of each chapter there is a comprehensive and useful bibliography. The author's index, plant index and general index at the end of the book enhance its utility. The book is profusely illustrated and most of the illustrations have been selected from the original publications. Mention may also be made of a few slips and printing mistakes which require to be rectified. By way of example, on page 287, the figure is intended to show lemna colouration but there are no colours in it ; on page 623 " Sabnis and Phatak " are wrongly spelt as " Sabris and Phatax " On page 899, " Lyallpur " is mis-spelt as " Syallpur " and " Afzal and Iyer " are noted as " Afzal and Tyer ".

The book is a useful contribution to the knowledge of science of applied botany and deserves to be read by every student interested in that science and should be on the shelf of every library as a reference book. (R.L.S.)

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